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Part 1. Protocols

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MASTER ANALYTICAL SCHEME FOR ORGANIC COMPOUNDS IN WATER:

PART 1. PROTOCOLS

by

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16. ABSTRACT

A Master Analytical Scheme (MAS) has been developed for the analysis of volatile (gas chromatographable) organic compounds in water. In developing the MAS, it was necessary to evaluate and modify existing analysis procedures and develop new techniques to produce protocols that provide for the comprehensive qualitative-quantitative analysis of almost all volatile organics in many types of water. The MAS provides for analysis of purgeable and extractable, as well as neutral and ionic water soluble, organics in surface and drinking waters and in leachates and various effluents. Nominal lower quantifiable limits range from 0.1 µg/L to 100 µg/L, depending on chemical/physical class of the analyte and complexity of the aqueous matrix. Recoveries are reported for about 280 model compounds of a wide variety of chemical classes and physical properties dosed into representative samples of several major types of water.

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FOREWORD

Nearly every phase of environmental protection depends on a capability to identify and measure specific contaminants in the environment. As part of this Laboratory's research on the occurrence, movement, transformation, impact and control of environmental contaminants, the Analytical Chemistry Branch characterizes chemical constituents of water and soil.

Chemists working in governmental, industrial, and academic laboratories have long recognized a need for a comprehensive analytical methodology for organic compounds in water. To this end, research was begun in 1978 to develop a qualitative-quantitative scheme for the analysis of organics of all volatility classes (amenable to gas chromatography), of almost all functional groups, in almost any water sample. The result is the Master Analytical Scheme (MAS) for Organic Compounds in Water, a compilation of protocols that employ new techniques and modifications of existing analysis procedures. Users may apply the MAS as a set of protocols for a comprehensive analysis or use individual protocols separately for analysis of organic fractions of particular interest. Among its many applications are epidemiological studies, wasteload allocations, permit application evaluations, trends analyses, watershed management studies, exposure assessments, landfill hazard evaluations, and aqueous source characterizations.

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PREFACE

The Master Analytical Scheme (MAS) represents the first effort to develop a comprehensive qualitative-quantitative scheme for the analysis of organic compounds in water. The MAS is a set of analytical protocols that includes a broad scope of organics with a wide variety of functional groups and physical properties. These protocols provide for the GC/MS/computer analysis of the usual purgeable and extractable compounds, with the addition of various neutral and ionic water soluble compounds; in fact, the MAS is applicable to any compound that can pass unchanged through a gas chromatograph, or can be derivatized to do so. Recoveries have been determined from distilled and drinking water, industrial and municipal effluents, and, in some cases, surface water and energy effluents, so the protocols are expected to be applicable to most water types. One unique feature of the MAS is its comprehensiveness. Another is its qualitative-quantitative aspect: an extensive data base of mass spectrometer detector response and recovery factors allows computer estimation of concentration without recourse to standards for each analyte.

In developing the MAS, existing analytical techniques were evaluated and modified and new techniques were developed to produce the comprehensive protocols. Development was in two stages. An interim set of protocols was developed by October 1980; analysis of environmental samples by these protocols revealed several important deficiencies that were subsequently corrected by additional experimental work. The final result is this first edition of MAS protocols.

Two companion reports resulted from MAS development: Experimental Development of the MAS for Organic Compounds in Water, and Literature Review for Development of the MAS for Organic Compounds in Water. The user can refer to the experimental development report for information on techniques considered for MAS incorporation and experiments dealing with technique optimization and recovery studies. The literature review, which covers material through June 1982 on techniques for analysis of organics in water, was the starting point for experimental development, and will also be of interest to many users. Neither companion report is essential to MAS use, however; this report (Part I: Protocols and Part II: Appendices to Protocols) stands alone as the handbook for implementation. Part II includes: Appendix A - specific instructions on fabrication of the purge and trap apparatus and ancillary devices for purgeable organics, Appendix B - hard copy of computerized relative molar response and recovery data for standards and analytes, and Appendix C - documentation of MASQUANT computer program for quantification of MAS data.

The prospective MAS user should thoroughly familiarize himself with Chapters 1 and 13 of this report (the Introduction and GC-MS-COMP Analysis Procedures) for an overview and a guide to use of the protocols.

Applications

The MAS was developed for two general types of application:

(1) The analysis of carefully selected samples to answer the question, "What compounds are present above detection limits and approximately how much of each is present?" It has been demonstrated repeatedly that one cannot predict reliably what chemicals will be found in surface waters, drinking water, or even industrial effluents for established manufacturing processes (Donaldson, W. T., Env. Sci. and Technol., 11, 348-351, 1977). Therefore, a strong need exists for broad spectrum identification and measurement methods for organic chemicals in such water. Applying the MAS should be cost effective in areas such as:

(a) Drinking Water - in epidemiological studies and as early warning for toxic pollutants below acutely toxic levels.

(b) Industrial Wastewaters - in wasteload allocations, permit application evaluation and long-range projections for the state of the environment.

(c) Surface Waters - in trends analysis, assessments of abatement program effectiveness, watershed management (including exposure assessment), and incident investigation.

(d) Landfill Leachates - in exposure assessment, evaluation of landfill performance, and diagnosis of problems.

(e) Environmental Processes - In chemical characterization of aqueous sources as related to fate and transport of organic compounds.

The complete set of MAS protocols may be applied for a survey analysis, or each protocol may be used as a separate entity for analysis of an organic fraction (e.g., purgeable volatiles) of particular interest.

(2) The second application addresses the need for repetitive analysis of selected pollutants for which methodology has not been developed. In the qualitative analysis of most samples that contain chemicals at high enough levels to merit further consideration, several pollutants will be identified for which methods have not been suggested (the vast majority of organic compounds, in fact, falls into this category). By applying only the part(s) of the MAS that relate to the pollutant(s) of interest, repetitive measurements for these pollutants can be made at a modest cost prior to the development of optimized new methods. In such applications a simpler selective detector can usually be substituted for the mass spectrometer.

ABSTRACT

A Master Analytical Scheme (MAS) has been developed for the analysis of volatile (gas chromatographable) organic compounds in water. In developing the MAS, it was necessary to evaluate and modify existing analysis procedures and develop new techniques to produce protocols that provide for the comprehensive qualitative-quantitative analysis of almost all volatile organics in many types of water. The MAS provides for analysis of purgeable and extractable, as well as neutral and ionic water soluble, organics in surface and drinking waters and in leachates and various effluents. Nominal lower quantifiable limits range from 0.1 µg/L to 100 µg/L, depending upon chemical/physical class of the analyte and complexity of the aqueous matrix. Recoveries are reported for about 280 model compounds of a wide variety of chemical classes and physical properties dosed into representative samples of several major types of water.

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CHAPTER 1

INTRODUCTION

This set of analytical protocols for the Master Analytical Scheme (MAS) is predicated on gas chromatography/mass spectrometry/computer (GC/MS/ COMP) as the final instrumental technique. As such, the MAS is designed for a comprehensive qualitative/quantitative analysis of purgeable, extractable, and water soluble organics in drinking and surface waters, municipal, industrial and energy-related effluents, and leachates. Although designed to span the complexity encountered in these water types, procedures are included that define the water quality and allow for optimal detection limits for that water sample. If the nominal detection limit for qualitative GC/MS analysis is assumed to be 10 ng for an organic compound, then the limits for the MAS range from 0.1 ppb (e.g., volatile organics in drinking water) to 100 ppb (e.g., nonvolatile strong acids in energy effluents) depending upon the chemical/physical class of the analyte and complexity of the matrix. In general, the quantitative limits of detection for target compounds with known retention times may be 5-10 times lower. More information on limits of detection are given in the introduction of each MAS operational protocol.

The prospective user has the latitude of applying all the protocols or just those that cover organic group types of interest. Thus, each protocol stands alone, containing the elements for determining water quality, collecting the sample, adding internal standards and processing the sample with subsequent analysis according to prescribed GC/MS/COMP conditions.

The purpose of this introduction is to provide an overview of the current MAS analytical operations and techniques, with recovery data for about 280 model compounds used during MAS development. This section also serves as a guideline for determining which protocols apply to specific classes or groups of compounds of interest.

1.1 ORGANIZATION OF MAS PROTOCOLS

Table 1.1 shows how these MAS protocols are organized and related to material in this introduction, and explains some MAS terminology. An understanding of the physical/chemical classifications of organics and how these relate to the analytical operational protocols and to the chapters in this volume is necessary for application of the MAS to a water sample. Symbols are for convenience in identifying each major MAS fraction, corresponding to a chemical/physical property group. Operational protocols in Table 1.1 are brief descriptions of the techniques for separation of organics from the water sample. Column 4 of Table 1.1 lists the tables, included in this introductory chapter, that provide recovery data for the applicable chemical classes and individual analytes for each protocol. These are the approximately 280 analytes for which recovery data were generated during development of the MAS. Finally, Table 1.1 lists the MAS chapters that are pertinent to analysis of each major MAS fraction. Generally, protocols for each fraction are divided into two chapters: one for sampling and sample handling and one for separation of the organics from water and analysis. Chapter 13 includes general GC/MS/COMP procedures common to all fractions.

The MAS user should study this introduction for an overview of the entire analytical scheme. If he is interested in analyzing for compounds contained in one or only a few protocols, he should refer to Table 1.1, which will lead him to the appropriate recovery tables (of 1.3-1.9). Examination of the chemical classes and individual analytes in these tables will indicate the appropriate protocols to apply. The tables give recoveries for all analytes tested. In several cases, recoveries are unacceptably low (<40%). Inclusion of these analytes serves to bracket the limits of compound types amenable to each protocol. Compounds whose recovery is indicated as unacceptable by one protocol are usually listed in another table at greater than 40%, indicating acceptable recovery by the other protocol. Recovery data, as well as relative molar response (RMR) factors, are also given in Appendix B (and in the computer data bank of MASQUANT). Use of Appendix B values and MASQUANT for quantitation are described in Chapter 13.

Table 1.1. ORGANIZATION OF MAS PROTOCOLS

MAS Symbol	Chemical/Physical Property Group	Operational Protocol	Example Classes and Analytes and Recoveries[a]	Pertinent MAS Chapters and Subjects
VO (volatile organics)	volatile, neutral, low water solubility	purge and trap	Table 1.3	2. Sampling and Sample Handline for VO 6. Purge, Trap and Analysis of VO
NEWS (neutral water soluble)	volatile, neutral, high water solubility	elevated temperature purge and trap	Table 1.4	3. Sampling and Sample Handling for NEWS 7. Elevated Temperature Purge, Trap, and Analysis of NEWS
WABN (weak acids and bases, and neutrals)	pH 8 extractable weak acids, weak bases, and neutrals	sorbent (accumulator) column partitioning (pH 8.0) (WABN-SC)	Table 1.5	4. Sampling and Sample Handling for Extractable Organics 9. Extraction and Analysis of WABN I. Sorbent Accumulator Column V. GC/MS/COMP
		batch liquid-liquid partitioning (pH 8.0) (WABN-BL)	Table 1.5	4. Sampling and Sample Handling for Extractable Organics 9. Extraction and Analysis of WABN II. Batch Liquid-Liquid IV. Clean-up V. GC/MS/COMP
		flow-under liquid-liquid partitioning (pH 8.0) (WABN-FU)	Table 1.5	4. Sampling and Sample Handling for Extractable Organics 9. Extraction and Analysis of WABN III. Flow-Under V. GC/MS/COMP
ESSA (extractable semivolatile strong acids)	pH 1 extractable, semivolatile strong acids	batch liquid-liquid partitioning (pH 1.0)	Table 1.6	4. Sampling and Sample Handling for Extractable Organics 8. Batch Liquid-Liquid Extraction and Analysis of ESSA
VOSA (volatile strong acids)	volatile carboxylic acids	anion exchange/distillation	Table 1.7	5. Sampling and Sample Handling for Other Ionic Compounds 10. Anion-Exchange, Distillation, and Analysis of VOSA
NOVA (nonvolatile acids)	very strong, nonextractable, nonvolatile acids	anion exchange	Table 1.8	5. Sampling and Sample Handling for Other Ionic Compounds 11. Anion-Exchange and Analysis of NOVA
SAM (strong amines)	primary and tertiary strong amines (SAM-PT)	cation exchange	Table 1.9	5. Sampling and Sample Handling for Other Ionic Compounds 12. Cation-Exchange and Analysis of SAM
	secondary strong amines (SAM-S)			
-	all groups	-	-	13. GC/MS/COMP Analysis Procedures - General Instructions for All Protocols

[a] Table in Chapter 1.

If specific compounds of interest are not found in the recovery tables, the user must look for similar compounds in the tables to decide which protocols to apply (see Section 1.4 - Summary of Recovery and Precision for MAS Protocols). He will then need to use chemical judgment in selection of recovery and RMR factors of structurally similar compounds from corresponding parts of Appendix B to apply towards estimates of analyte concentrations. More guidance in this procedure is given in Chapter 13.

1.2 MAS OVERVIEW

Figure 1.1 depicts a flow diagram of the procedures for implementation of the MAS. Each step is summarized below.

1.2.1 Sample Handling

Seven sub-samples are required for a comprehensive sample analysis: one for each protocol class. Procedures are prescribed in the protocols for sample collection, storage and preservation. Volatile organic (VO fraction) samples are collected in septum-capped bottles with no headspace. Methylene chloride is added to all extractable and ionic compound samples as a bacteriocide, while hexane is used as "keeper" solvent layer for extractable compounds. Chlorine determination indicates the level of sodium thiosulfate necessary to stoichiometrically reduce any residual chlorine left from water treatment. All samples are stored at ~4°C in the dark.

Various water quality scouting measurements help in the selection of appropriate analytical procedures, which are optimized according to water quality rather than sample "type" (e.g., drinking water or municipal effluent). Headspace gas analysis by GC of a separate small sample is employed to determine the dilution necessary for VO purge and trap analysis. A trial shake-out with methylene chloride of a small aliquot of the extractable (WABN) sample shows whether emulsion formation is a problem, and thus whether the flow-under extractor must be used. Conductivity measurements indicate maximum sample volume allowable for isolation of ionic compounds by ion-exchange resin without exceeding resin capacity.

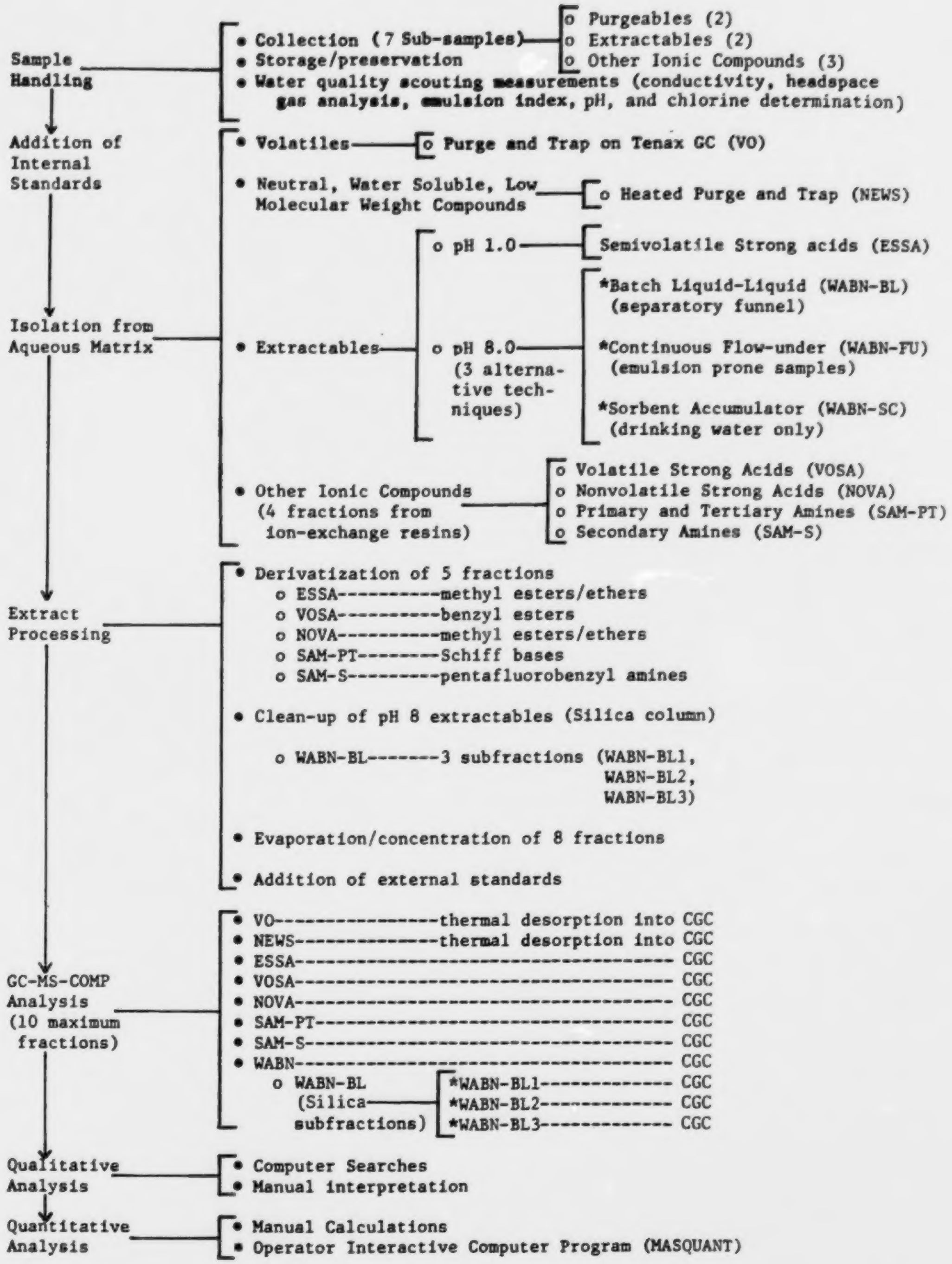
Sample Handling
• Collection (7 Sub-samples)
o Purgeables (2)
o Extractables (2)
o Other Ionic Compounds (3)
• Storage/preservation
• Water quality scouting measurements (conductivity, headspace gas analysis, emulsion index, pH, and chlorine determination)
Addition of Internal Standards
• Volatiles
o Purge and Trap on Tenax GC (VO)
• Neutral, Water Soluble, Low Molecular Weight Compounds
o Heated Purge and Trap (NEWS)
Isolation from Aqueous Matrix
• Extractables
o pH 1.0
Semivolatile Strong acids (ESSA)
o pH 8.0 (3 alternative techniques)
*Batch Liquid-Liquid (WABN-BL) (separatory funnel)
*Continuous Flow-under (WABN-FU) (emulsion prone samples)
*Sorbent Accumulator (WABN-SC) (drinking water only)
• Other Ionic Compounds (4 fractions from ion-exchange resins)
o Volatile Strong Acids (VOSA)
o Nonvolatile Strong Acids (NOVA)
o Primary and Tertiary Amines (SAM-PT)
o Secondary Amines (SAM-S)
Extract Processing
• Derivatization of 5 fractions
o ESSA----------methyl esters/ethers
o VOSA----------benzyl esters
o NOVA----------methyl esters/ethers
o SAM-PT--------Schiff bases
o SAM-S---------pentafluorobenzyl amines
• Clean-up of pH 8 extractables (Silica column)
o WABN-BL-------3 subfractions (WABN-BL1, WABN-BL2, WABN-BL3)
• Evaporation/concentration of 8 fractions
• Addition of external standards
GC-MS-COMP Analysis (10 maximum fractions)
• VO----------------thermal desorption into CGC
• NEWS--------------thermal desorption into CGC
• ESSA-- CGC
• VOSA-- CGC
• NOVA-- CGC
• SAM-PT-------------------------------------- CGC
• SAM-S--------------------------------------- CGC
• WABN--- CGC
o WABN-BL (Silica subfractions)
*WABN-BL1------------- CGC
*WABN-BL2------------- CGC
*WABN-BL3------------- CGC
Qualitative Analysis
• Computer Searches
• Manual interpretation
Quantitative Analysis
• Manual Calculations
• Operator Interactive Computer Program (MASQUANT)

Figure 1.1. Master Analytical Scheme Flow Diagram.

1.2.2 Internal Standards

Prescribed deuterated internal standards (Table 1.2) are added to each sub-sample before processing or storage, preferably in the field. Selection and packaging assures that from one to nine standards of the total of 20 will appear in each extract for GC/MS analysis; retention times are such that the standards span the chromatographic window in most cases. These standards are used for monitoring recovery during analysis, for quantifying sample components, and for relative retention time calculations.

The initial sets of MAS standards were prepared by the National Bureau of Standards (NBS). After assuring chemical and isotopic purity of each standard compound, NBS determined its stability and compatability for packaging in solution with other internal standards. Methanol solutions (water solutions for highly water-soluble compounds) were prepared and packaged for sample dosing in the combinations shown in Table 1.2.

Purgeable NBS standards are packaged in glass capillary ampoules that are placed in the sample bottle and crushed with a magnetic stirbar after the water sample has been collected. For other sample aliquots, internal standards are packaged in vials such that emptying the entire content of the vial into the prescribed sample volume produces the concentrations of standards shown in Table 1.2. Low concentration standards sets are generally used for drinking and surface waters. Higher concentration sets are used for effluents.

1.2.3 Isolation of Organics

After addition of internal standards, the seven subsamples are processed as follows. (Protocol symbols are in parenthesis.)

1.2.3.1 Volatile Organics (VO)--

Highly volatile (purgeable) organics (Table 1.3) are analyzed by a modification of the Bellar-Lichtenberg (EPA's Method 624) method, using a custom built purge and trap system (Appendix A) designed especially for capillary GC columns. Sodium sulfate is used to "salt out" the organics in a 200 mL sample, which is purged at 30°C. More concentrated samples are first diluted to 200 mL in accordance with the total concentration of purgeable organics as indicated by GC scouting of the headspace. Dilution prevents saturation of the GC/MS/COMP and decreases foaming potential.

Table 1.2. INTERNAL AND EXTERNAL STANDARDS EMPLOYED IN THE MASTER ANALYTICAL SCHEME

MAS Symbol	Operational Protocol	Internal Standards	µg/L[a] Low	µg/L[a] High	External Standard
VO (volatile organics)	Purge and Trap	bromoethane-d_5	0.56	6.6	perfluorotoluene
		anisole-2,4,6-d_3	4.2	25.6	
		chlorobenzene-d_5	7.2	5.4	
		naphthalene-d_8	5.8	48.7	
NEWS (neutral water soluble)	Elevated Temperature Purge and Trap	*t*-butanol-d_9	112.9	554.0	perfluorotoluene
		nitrobenzene-d_5	20.1	103.5	
WABN (weak acids, bases and neutrals)	Sorbent Column, Batch L-L, or Flow-under Partitioning (pH 8.0)	*o*-xylene-d_{10}	2.8	104.7	4-fluoro-2-iodotoluene
		naphthalene-d_8	20.2	500.7	
		nitrobenzene-d_5	94.2	538.8	
		1-phenyl-d_5-ethanol	--	520.9	
		acetophenone-d_5	--	104.7	
		propiophenone-d_5	--	98.1	
		perylene-d_{12}	1.1	98.1	
		acridine-d_9	19.8	100.3	
		phenol-d_5	112.7	400.4	
ESSA (extractable semi-volatile strong acics)	Batch L-L Partitioning (pH 1.0)	heptanoic acid-d_{13}	13.8	111.0	4-fluoro-2-iodotoluene
		benzoic acid-d_5	1.08	100.3	
VOSA (volatile strong acids)	Anion-exchange/ Distillation	butyric acid-d_7	15.7	535.9	2-fluorobiphenyl
NOVA (nonvolatile acids)	Anion-exchange	2-naphthalenesulfonic acid-$d_7 \cdot H_2O$	99.4	497.5	2-fluorobiphenyl 4-fluoro-2-iodotoluene
SAM (strong amines)	Cation-exchange	*n*-butylamine-d_9	118.3	554.3	2-fluorobiphenyl
		2-phenylethyl-1,1,2,2-d_4-amine	121.2	530.3	4-fluoro-2-iodotoluene
		N-ethyl-2-fluorobenzyl-amine	107.2	522.1	2-fluorobiphenyl 4-fluoro-2-iodotoluene

[a] Concentration after dosing into the appropriate volume of sample. These concentrations are based upon the "400 series" production run of internal standard solutions prepared by NBS.

Table 1.3. EXAMPLE CLASSES AND ANALYTES RECOVERED BY THE PURGE AND TRAP ANALYTICAL PROTOCOL (VO)

- Volatility range ~-13°C to 220°C BP
- Slightly soluble to nonsoluble in water
- Relatively nonpolar

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)	Overall Recoveries[a] Mean ± S.D. (C.V.)
Aromatic Hydrocarbons	benzene	78 ± 6 (7)	76 ± 3 (4)
	toluene	125 ± 6 (5)	125 ± 6 (5)[b]
	p-xylene	108 ± 13 (12)	98 ± 13 (14)
	ethylbenzene	96 ± 5 (5)	96 ± 7 (7)
	1,3,5-trimethylbenzene	65 ± 3 (5)	77 ± 18 (23)
	1,2,4-trimethylbenzene	97 ± 2 (2)	94 ± 4 (5)
	t-butylbenzene	113 ± 3 (3)	113 ± 3 (3)[b]
	sec-butylbenzene	-[d]	113 ± 3 (3)
	o-diethylbenzene	98 ± 6	-
	p-diethylbenzene	61 ± 4 (6)	81 ± 28 (35)
	4-methylisopropylbenzene	100 ± 5 (5)	100 ± 5 (5)[b]
	naphthalene	-	74 ± 2 (3)
	diphenylmethane	-	59 ± 5 (9)
Halogenated Aromatics	fluorobenzene	116 ± 5 (5)	116 ± 5 (5)[b]
	chlorobenzene	98 ± 10 (10)	91 ± 15 (15)
	bromobenzene	123 ± 12 (10)	123 ± 12 (10)[b]
	p-bromotoluene	104 ± 4 (3)	104 ± 4 (3)
	p-iodotoluene	96 ± 8 (8)	106 ± 15 (14)
	1,2-dichlorobenzene	116 ± 10 (9)	116 ± 10 (9)[b]
	1,3-dichlorobenzene	90 ± 4 (5)	103 ± 18 (18)
	1,4-dichlorobenzene	83 ± 4 (4)	83 ± 4 (4)[b]
	2-bromochlorobenzene	73 ± 3 (4)	92 ± 27 (30)

(continued)

Table 1.3 (cont'd.)

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)	Overall Recoveries[a] Mean ± S.D. (C.V.)
	4-bromochlorobenzene	-	106 ± 2 (2)
	1,2,4-trichlorobenzene	88 ± 3 (3)	101 ± 21 (21)
	α,α,α-trichlorotoluene	71 ± 11 (16)	70 ± 11 (16)[b]
Misc. Aromatics	anisole	62 ± 1 (2)	68 ± 8 (12)
	phenyl ether	-	47
Aliphatic and Alicyclic Hydrocarbons	cyclopentane	95 ± 4 (5)	113 ± 25 (22)
	cyclohexane	79 ± 8 (10)	85 ± 8 (10)
	n-hexane	90 ± 17 (19)	105 ± 21 (20)
	n-heptane	-	120 ± 4 (3)
	n-octane	103 ± 11 (11)	103 ± 1 (1)
	n-nonane	80 ± 32 (40)	79 ± 32 (40)
	n-decane	70 ± 10 (14)	89 ± 9 (10)
	n-undecane	-	50 ± 4 (8)
	n-dodecane	64	67 ± 5 (7)
	n-tridecane	52 ± 18 (34)	44 ± 11 (26)
	n-tetradecane	58 ± 22 (38)	49 ± 13 (26)
	dipentene	96 ± 15 (16)	96 ± 15 (16)[b]
Halogenated Aliphatic Hydrocarbons	chloroform	74 ± 4 (6)	78 ± 6 (7)
	bromochloromethane	48 ± 3 (7)	95 ± 67 (71)
	trans-1,2-dichloroethylene	82 ± 6 (7)	87 ± 8 (9)
	1,2-dichloroethane	102 ± 10 (10)	102 ± 10 (10)[b]
	1,2-dibromoethane	68 ± 5 (8)	77 ± 13 (16)
	trichloroethylene	118 ± 9 (8)	118 ± 9 (8)
	allyl chloride	-	64 ± 12 (19)[b]
	tetrachloroethylene	82 ± 7 (8)	77 ± 6 (8)
	1,2-dichloropropane	-	128 ± 12 (10)[b]
	2-bromo-1-chloropropane	99 ± 4 (4)	99 ± 4 (4)[b]
	1,2-dibromopropane	90 ± 4 (4)	90 ± 4 (4)

(continued)

Table 1.3 (cont'd.)

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)	Overall Recoveries[a] Mean ± S.D. (C.V.)
Halogenated Aliphatic Hydrocarbons (continued)	2-bromobutane	117 ± 3 (3)	117 ± 3 (3)[b]
	1,4-dibromobutane	94 ± 7 (7)	103 ± 13 (13)
	1-chlorohexane	128 ± 11 (9)	128 ± 11 (9)[b]
	1-bromohexane	129 ± 7 (5)	129 ± 7 (5)[b]
Misc. O,S Compounds	propylene oxide	67 ± 15 (22)	67 ± 15 (22)[b]
	diethyl ether	-	115 ± 5 (4)
	2-methyl furan	65 ± 3 (5)	80 ± 21 (26)
	allyl ether	87 ± 9 (10)	98 ± 15 (16)
	hexyl ether	68 ± 16 (23)	70 ± 3 (4)
	carbon disulfide	-	100 ± 3 (3)
	thiophene	120 ± 7 (6)	120 ± 7 (6)[b]
Deuterated Standards	d_5-bromoethane	91 ± 20 (23)	85 ± 16 (19)
	2,4,6-d_3-anisole	77 ± 11 (15)	57 ± 15 (25)
	d_5-chlorobenzene	109 ± 6 (6)	120 ± 20 (16)
	d_8-naphthalene	-	99 ± 10 (10)[c]

[a] The recovery is a mean value for triplicate determinations in drinking water, spiked at 0.2 to 1.8 ppb (nominally 1 ppb), and triplicate determinations in a 60/40 industrial/municipal wastewater, spiked at 30 to 87 ppb (nominally 50 ppb). S.D. = standard deviation, C.V. = coefficient of variation.

[b] Recovery is for triplicate determination in drinking water only.

[c] Recovery is for triplicate determination in industrial/municipal wastewater only.

[d] Not determined.

Purged organic vapors are collected on a Tenax GC sorbent trap, from which they are thermally desorbed into a liquid nitrogen cold trap. Before desorption of the purged sample components into the cold trap, an "external" standard, perfluorotoluene, is added to the cold trap through an injection port system. The injection port is installed between the sorbent trap and the cold trap. The total condensate is then flash evaporated into a fused silica capillary for analysis by GC/MS/COMP. Comparison of MS signals for the external standard with those for the internal standards purged from the sample allows calculation of recoveries of the internal standards, thus monitoring performance of the entire analytical operation.

1.2.3.2 Neutral Water Soluble Compounds (NEWS)--

Low molecular weight, water soluble, non-extractable compounds (Table 1.4) are purged from a 10 mL water sample containing 20% sodium chloride at 80°C and trapped on Tenax, using the same equipment as for the VO fraction. To achieve lower limits of detection for drinking water, a 200 mL sample is concentrated by azeotropic distillation in a Peters' (Peters, T. I., Anal. Chem., *52*, 211, 1980) apparatus to produce a 3 mL aqueous condensate enriched in neutral organics. This condensate is then purged as above.

1.2.3.3 Organics Extracted at pH 8 (WABN)--

Compounds of intermediate volatility, most of which are water insoluble (Table 1.5), are analyzed by batch liquid-liquid extraction of 1 L of water sample with methylene chloride in a separatory funnel. Adjustment of sample pH to 8.0 allows reproducible extraction of the weak acids, (*e.g.*, most phenols) and weak bases, (*e.g.*, most anilines) as well as neutral compounds.

For some samples, however, batch liquid-liquid extraction is not suitable. An initial trial solvent extraction in a stoppered graduated cylinder indicates whether emulsion formation is likely to be a problem. For emulsion-prone samples, continuous liquid-liquid extraction with methylene chloride in a flow-under extractor should be used. For samples in which extractable organic concentration is expected to be low, such as drinking water and some surface waters, XAD-4 resin sorbent accumulator columns are used for sorption/concentration from 10-15 L of water. The organics are desorbed using methanol followed by methylene chloride.

Table 1.4. EXAMPLE CLASSES AND ANALYTES RECOVERED BY THE ELEVATED TEMPERATURE PURGE AND TRAP PROTOCOL (NEWS)

- Very soluble to slightly water soluble
- Volatility range ~35°C to 225°C BP
- Relatively Polar

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)	Overall Recoveries^{a} Mean ± S.D. (C.V.)
Alcohols	1-propanol	104 ± 4 (4)	108 ± 19 (18)
	1-butanol	120 ± 12 (10)	120 ± 12 (10)^{b}
	1-pentanol	129 ± 14 (10)	113 ± 22 (20)
	1-hexanol	117 ± 13 (11)	131 ± 32 (25)
	1-heptanol	94 ± 10 (11)	106 ± 25 (24)
	1-octanol	100 ± 11 (11)	100 ± 11 (11)^{b}
	cyclohexanol	122 ± 10 (8)	122 ± 10 (8)^{c}
	furfuryl alcohol	-^{d}	64 ± 10 (16)^{c}
Aldehydes	propionaldehyde	99 ± 41 (41)	99 ± 41 (41)^{b}
	n-butyraldehyde	53 ± 17 (32)	72 ± 23 (32)
	n-valeraldehyde	93 ± 27 (29)	81 ± 23 (29)
	crotonaldehyde	81 ± 20 (25)	83 ± 21 (25)^{c}
	furfural	-	36 ± 6 (16)^{c}
Esters	methyl formate	25 ± 13 (54)	45 ± 4 (8)^{c}
	methyl acetate	-	93 ± 5 (6)^{c}
	ethyl acetate	35 ± 7 (19)	89 ± 5 (6)^{c}
	n-propyl acetate	40 ± 8 (20)	79 ± 4 (5)^{c}
	allyl acetate	51 ± 9 (18)	79 ± 13 (17)^{c}
	n-butyl acetate	34 ± 11 (33)	72 ± 4 (5)^{c}
	ethyl butyrate	27 ± 9 (35)	63 ± 2 (4)^{c}

(continued)

Table 1.4 (cont'd.)

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)	Overall Recoveries[a] Mean ± S.D. (C.V.)
Ethers	tetrahydrofuran	62 ± 14 (23)	79 ± 25 (31)
	dioxane	23 ± 0.5 (2)	50 ± 7 (14)[c]
Ketones	methyl ethyl ketone	53 ± 20 (34)	69 ± 22 (32)
	cyclopentanone	52 ± 6 (11)	52 ± 6 (11)[b]
	cyclohexanone	65 ± 3 (4)	65 ± 1 (2)
Nitriles	acrylonitrile	30 ± 9 (31)	89 ± 5 (6)[c]
	propionitrile	95 ± 6 (7)	95 ± 6 (7)
	isobutyronitrile	62 ± 7 (12)	57 ± 7 (14)
	benzonitrile	90 ± 8 (9)	91 ± 8 (9)
Nitro Compounds	nitromethane	67 ± 3 (4)	70 ± 5 (7)
	1-nitropropane	84 ± 8 (9)	93 ± 13 (14)
	nitrobenzene	92 ± 9 (10)	96 ± 6 (7)
Deuterated Standards	d_9-t-butanol	93 ± 21 (23)	98 ± 7 (7)
	d_5-nitrobenzene	87 ± 12 (14)	93 ± 9 (10)

[a] Recovery is a mean value for triplicate determinations from drinking water, spiked at 0.8 to 1.2 ppb (nominally 1 ppb), and for triplicate determinations from a 60/40 industrial/ municipal wastewater, spiked at 40 to 63 ppb (nominally 50 ppb). S.D. = standard deviation, C.V. = coefficient of variation.

[b] Mean recovery for triplicate determinations from drinking water only.

[c] Mean recovery for triplicate determinations from wastewater only.

[d] No data.

Table 1.5. EXAMPLE CLASSES AND ANALYTES RECOVERED BY ACCUMULATOR COLUMN AND BATCH L-L PARTITION PROTOCOLS AT pH 8.0 (WABN)

Applicable Chemical Classes	Physical/Chemical Properties Encompassed	Example Analytes	Overall Recovery [Mean ± S.D. (C.V.)][a]		
			Accumulator Column (WABN-SC)[b]	Batch L-L (WABN-BL)[c]	
				No Clean-up	With Clean-up[d]
Weak Acids	• $pK_a < 7$ • B.P. ~115° up to limit of chromatography	phenol	55 ± 6 (11)	-[e]	61 ± 11 (18)**+
		p-cresol	63 ± 15 (23)	72 ± 32 (44)	62 ± 27 (44)**+
		2,3-dimethylphenol	95 ± 5 (5)	-	58 ± 14 (24)**
		o-isopropylphenol	88 ± 9 (10)	74 ± 7 (9)	57 ± 5 (9)**+
		o-nitrophenol	-	-	49 ± 10 (22)**+
		6-chloro-2-methylphenol	90[f]	98 ± 43 (44)	118 ± 11 (10)**
		1-naphthol	-	-	89 ± 18 (20)**
		p-t-butylphenol	86 ± 5 (6)	80 ± 13 (16)	77 ± 6 (8)**
		2-nitro-p-cresol	32 ± 17 (53)	76 ± 16 (21)	61 ± 9 (15)**
		2,4-dichlorophenol	78	-	81 ± 26 (32)**
		2,4,6-trichlorophenol	66	-	30 ± 4 (12)*+
		di-t-butyl-4-methylphenol	73	-	69 ± 14 (20)**
		4-chloro-3-methylphenol	76 ± 7 (9)	-	66 ± 9 (14)**+
Weak Bases	• $pK_b > 3.5$ • B.P. ~115° up to limit of chromatography	pyridine	-	58 ± 24 (42)	-
		α-picoline	36 ± 4 (11)	66 ± 17 (26)	49 ± 13 (26)**+
		aniline	34 ± 11 (32)	84 ± 34 (40)	63 ± 25 (40)**+
		lutidine	95 ± 6 (6)	57 ± 31 (54)	-
		indole	95 ± 6 (6)	86 ± 36 (42)	44 ± 13 (30)**
		2,3,6-trimethylpyridine	84 ± 1 (1)	82 ± 25 (31)	60 ± 21 (35)**
		2,6-dimethylaniline	87 ± 10 (11)	72 ± 6 (8)	86 ± 18 (21)**
		m-chloroaniline	95 ± 5 (5)	88 ± 25 (28)	80 ± 5 (6)**
		quinoline	91 ± 13 (14)	77 ± 24 (31)	53 ± 16 (31)**+
		p-nitroaniline	77 ± 4 (5)	-	-
		2,4-dimethylquinoline	90 ± 1 (1)	80 ± 19 (24)	55 ± 20 (36)**
		nicotine	62 ± 10 (16)	84 ± 17 (20)	-
		carbazole	82 ± 2 (2)	79 ± 8 (10)	73 ± 16 (22)**
		2-aminobiphenyl	82 ± 8 (8)	91 ± 15 (17)	55 ± 13 (24)*+
		diphenylamine	85 ± 1 (1)	76 ± 20 (27)	87 ± 11 (13)**
		tributylamine	82 ± 0 (0)	70 ± 17 (25)	40 ± 10 (25)**+
		caffeine	86 ± 5 (6)	92 ± 16 (17)	68 ± 12 (18)**+
		dibenzylamine	53 ± 8 (15)	62 ± 18 (29)	43 ± 12 (29)**+
		N,N-dimethyldodecylamine	69	68 ± 14 (20)	74 ± 13 (18)**
		atrazine	71 ± 7 (10)	90 ± 5 (6)	78 ± 12 (16)**+
Alkanes	• B.P. ~150°C up to limit of chromatography • Water/CH_2Cl_2 partition coefficient ≤1	n-decane		64 ± 26 (41)	
		n-tridecane	44 ± 8 (18)	66 ± 15 (23)	51 ± 12 (23)*+
		n-tetradecane	42 ± 7 (17)	48 ± 12 (25)	45 ± 11 (25)*+
		n-pentadecane	-	68 ± 14 (20)	62 ± 12 (20)*+
		n-hexadecane	45 ± 5 (11)	51 ± 15 (29)	51 ± 15 (29)*+
		n-heptadecane	-	78 ± 10 (13)	77 ± 10 (13)*+
		n-octadecane	46 ± 3 (6)	67 ± 15 (22)	66 ± 14 (22)*+
		n-nonadecane	47 ± 3 (6)	63 ± 11 (17)	65 ± 11 (17)*+
		n-eicosane	8 ± 0 (0)	75 ± 23 (31)	82 ± 25 (31)*+
		n-heneicosane	63 ± 12 (19)	66 ± 12 (18)	65 ± 12 (18)*+
		n-docosane	66 ± 13 (20)	64 ± 12 (19)	59 ± 11 (19)*+
		n-tricosane	66 ± 13 (20)	65 ± 11 (17)	58 ± 10 (17)*+

(continued)

Table 1.5 (cont'd.)

Applicable Chemical Classes	Physical/Chemical Properties Encompassed	Example Analytes	Overall Recovery [Mean ± S.D. (C.V.)][a]		
			Accumulator Column (WABN-SC)[b]	Batch L-L (WABN-BL)[c]	
				No Clean-up	With Clean-up[d]
Aliphatic Ketones, Alcohols, and Esters	"	2-heptanone	66	-	55 ± 5 (10)**
		butoxyethanol	-	65 ± 16 (24)	70 ± 10 (15)**+
		2-octanone	77	70 ± 10 (14)	55 ± 15 (27)**+
		butyl propionate	52 ± 10 (20)	61 ± 13 (21)	31 ± 6 (21)**+
		isophorone	85 ± 4 (5)	78 ± 16 (21)	71 ± 25 (35)**
		fenchone	64 ± 14 (22)	74 ± 23 (31)	63 ± 19 (31)**+
		α-terpineol	92 ± 1 (1)	75 ± 24 (32)	111 ± 27 (24)**
		n-decanol	94 ± 1 (1)	95 ± 39 (41)	71 ± 20 (28)**
		dihydrofuran	89 ± 2 (2)	-	-
		dimethyl adipate	91	62 ± 23 (43)	49 ± 21 (43)**+
		methyl stearate	49 ± 8 (16)	77 ± 14 (18)	91 ± 14 (15)**
Misc. Aliphatic Compounds	"	1,4-dicyanobutane	70 ± 29 (36)	135 ± 38 (28)	104 ± 37 (36)**
		butyl carbamate	40 ± 4 (10)	60 ± 16 (26)	-
		di-t-butyldisulfide	58 ± 10 (17)	-	57 ± 6 (11)*
		bis(2-chloroethoxy)ethane	92 ± 11 (12)	78 ± 26 (33)	65 ± 17 (26)**
		tributylphosphate	86 ± 2 (2)	93 ± 10 (11)	75 ± 7 (9)**
		aldrin	83 ± 23 (28)	-	96 ± 3 (3)*+
Aromatic Hydrocarbons	"	2-methylnaphthalene	65 ± 18 (27)	71 ± 14 (19)	48 ± 9 (19)*+
		acenaphthene	76 ± 17 (22)	78 ± 21 (27)	50 ± 13 (27)*+
		biphenyl	78 ± 15 (19)	80 ± 17 (21)	-
		1,8-dimethylnaphthalene	78 ± 16 (20)	71 ± 13 (18)	48 ± 9 (18)*+
		fluorene	87 ± 15 (17)	85 ± 18 (21)	84 ± 12 (14)**
		2,3,5-trimethylnaphthalene	84 ± 22 (26)	74 ± 11 (15)	48 ± 6 (13)**
		anthracene	78 ± 1 (1)	-	66 ± 16 (24)**
		pyrene	73 ± 2 (3)	80 ± 7 (9)	78 ± 19 (24)**
		9,10-dimethylanthracene	60 ± 4 (7)	45 ± 21 (47)	63 ± 26 (41)**
		chrysene	43	78 ± 27 (35)	95 ± 15 (16)**
		perylene	62 ± 13 (20)	54 ± 13 (24)	118 ± 40 (34)**
Halogenated Aromatic Compounds	"	benzyl chloride	35 ± 2 (6)	63 ± 28 (45)	82 ± 14 (17)*
		4-chlorobenzonitrile	80 ± 9 (11)	79 ± 29 (37)	47 ± 12 (26)**
		3-chlorobenzaldehyde	-	-	80 ± 11 (14)**
		o-chloroanisole	57 ± 6 (10)	78 ± 29 (37)	50 ± 9 (18)**
		3,4-dichlorobenzaldehyde	100 ± 10 (10)	78 ± 28 (37)	55 ± 9 (17)**
		1,2,4-trichlorobenzene	71 ± 5 (7)	73 ± 13 (18)	107 ± 14 (13)**
		2-bromochlorobenzene	56 ± 19 (34)	-	-
		4-fluoro-2-iodotoluene	62 ± 26 (42)	82 ± 14 (17)	-
		1,2-dichloronaphthalene	87 ± 1 (1)	77 ± 25 (33)	68 ± 22 (33)**
		1,2,4,5-tetrachlorobenzene	77	69 ± 17 (25)	53 ± 13 (25)*+
		p-dibromobenzene	65 ± 12 (18)	80 ± 2 (3)	43 ± 7 (17)*
		4-bromophenyl phenyl ether	91 ± 14 (15)	80 ± 2 (3)	80 ± 18 (22)**
		hexachlorobenzene	64 ± 6 (9)	-	95 ± 6 (16)*+

(continued)

Table 1.5 (cont'd.)

Applicable Chemical Class(es)	Physical/Chemical Properties Encompassed	Example Analytes	Overall Recovery [Mean ± S.D. (C.V.)][a]		
			Accumulator Column (WABN-SC)[b]	Batch L-L (WABN-BL)[c]	
				No Clean-up	With Clean-up[d]
Aromatic Aldehydes and Ketones	"	benzaldehyde	-	89 ± 31 (35)	43 ± 6 (15)**
		o-tolualdehyde	88 ± 15 (17)	66 ± 24 (36)	105 ± 19 (18)**
		acetophenone	92 ± 2 (2)	-	-
		salicylaldehyde	-	73 ± 44 (60)	67 ± 17 (25)**
		anisaldehyde	96 ± 16 (17)	72 ± 10 (14)	62 ± 9 (14)**+
Aromatic Esters and Sulfonates	"	phenyl acetate	74	-	32 ± 6 (18)**
		benzyl acetate	79 ± 10 (13)	74 ± 12 (16)	55 ± 8 (14)**
		methylbenzene sulfonate	36 ± 6 (17)	81 ± 30 (37)	-
		methyl-p-toluene sulfonate	46 ± 8 (17)	67 ± 47 (70)	-
		diethylphthalate	87 ± 6 (7)	108 ± 7 (6)	89 ± 6 (7)**
		ethyl-p-toluene sulfonate	53 ± 4 (7)	91 ± 36 (40)	70 ± 18 (26)**
		dimethylphthalate	74 ± 8 (11)	107 ± 39 (37)	60 ± 7 (12)**
		dibutylphthalate	-	96 ± 10 (10)	138 ± 3 (2)**
		butylbenzylphthalate	64	97 ± 27 (28)	74 ± 13 (18)**
		diethylhexylphthalate	74 ± 12 (16)	102	104 ± 19 (18)**+
Misc. Aromatic Compounds	"	2,3-dihydrobenzofuran	-	67 ± 13 (20)	48 ± 9 (18)**
		nitrobenzene	83	86 ± 19 (22)	58 ± 13 (22)**+
		benzothiazole	85 ± 12 (14)	71 ± 8 (11)	58 ± 5 (8)**
		phenylcarbamate	-	-	48 ± 9 (18)**
		2,4-dinitrotoluene	89 ± 10 (11)	-	105 ± 11 (11)**
		benzylsulfide	67 ± 7 (10)	84 ± 6 (7)	71 ± 12 (17)**
		diphenylsulfone	69 ± 4 (6)	86 ± 1 (1)	67 ± 8 (12)**
		triphenylphosphate	84 ± 21 (24)	93 ± 29 (31)	71 ± 7 (10)**
		diphenylmercury	-	112 ± 12 (11)	81 ± 16 (20)**
		tetraphenyltin	47 ± 5 (11)	94 ± 19 (20)	72 ± 24 (33)**
Deuterated Internal Standards		d_{10}-xylene	76 ± 6 (8)	58 ± 13 (23)	46 ± 12 (26)*
		d_5-phenol	55 ± 6 (11)	85 ± 34 (40)	66 ± 26 (40)**+
		d_5-acetophenone	87 ± 10 (11)	75 ± 10 (14)	-
		d_5-phenylethanol	78 ± 8 (10)	80 ± 22 (27)	62 ± 20 (32)**
		d_5-nitrobenzene	82 ± 10 (12)	79 ± 13 (17)	55 ± 16 (29)**
		d_5-propiophenone	93 ± 4 (4)	73 ± 11 (16)	42 ± 8 (18)**
		d_8-naphthalene	79 ± 3 (4)	71 ± 9 (12)	40 ± 4 (11)**
		d_9-acridine	87 ± 14 (10)	84 ± 13 (15)	77 ± 11 (15)**+
		d_{12}-perylene	62 ± 13 (21)	80 ± 29 (36)	78 ± 28 (36)**+

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[a] The flow-under extraction protocol for emulsion-prone samples (WABN-FU) requires the determination of recoveries in the sample matrix under investigation, since it has been determined that large variations occur from one sample matrix to another. Therefore, no recoveries are given here. MASQUANT requires the user to input recovery factors as they are determined on these samples.

[b] Recoveries are for triplicate determinations from drinking water, spiked at 0.5 to 5 ppb (nominally 1 ppb).

[c] Recoveries are for triplicate determinations from a 60/40 industrial/municipal wastewater, spiked at 15 to 50 ppb (nominally 25 ppb).

[d] With clean-up step included; * = subfraction 1, ** = subfraction 2+3, (recoveries of compounds in subfraction 3 were not determined separately). **+ = subfraction 2+3, and *+ = subfraction 1 are from reagent water, interference in wastewater prevented recovery determination.

[e] - = not determined.

[f] Single determination.

1.2.3.4 Organics Extracted at pH 1 (ESSA)--

Extraction at pH 8 does not efficiently recover strong acids or bases. Strong bases are extracted on ion-exchange resins, but a new procedure has been developed for semivolatile strong acids (Table 1.6). This involves batch liquid-liquid extraction of a 1 L sample with methylene chloride at pH 10 to remove most neutrals and bases (discarded), after which the sample is made to pH 1.0 with HCl and the semivolatile strong acids are extracted with methyl-*t*-butyl ether. This procedure includes most carboxylic acids and strongly acidic phenols. The lower molecular weight carboxylic acids, however, are included in a separate volatile acids protocol (VOSA); they are too volatile to be efficiently recovered during liquid-liquid extraction and subsequent extract processing. In addition, some acids, *e.g.*, sulfonic acids, are too ionic to be extracted under these conditions and are included in the nonvolatile strong acid analytical protocol (NOVA).

This is followed by derivatization with diazomethane to form the corresponding methyl esters or ethers before GC/MS analysis.

1.2.3.5 Other Ionic Compounds (VOSA, NOVA, SAM)--

Compounds that are easily dissociated in water have not previously been included in analytical schemes because of difficulties with extraction and chromatography. New techniques, however, were developed to allow inclusion of most of these compounds in the MAS. Ion-exchange resins are used to separate four classes of ionic compounds from the sample matrix using three separate aliquots of the sample.

"Volatile" strong acids (VOSA), such as acrylic acid, octanoic acid, and other volatile carboxylic acids (Table 1.7) are separated from the water on Biorad AG 1-X8 anion exchange resin, then eluted with sodium bisulfate in an acetone:water solution. The volatile acids are distilled from the eluate, converted to nonvolatile salts, then derivatized with benzylbromide to form benzyl esters.

"Nonvolatile" strong acids (NOVA), e.g., naphthalene sulfonic acids, (Table 1.8) are also separated from water on Biorad AG 1-X8 resin. They are eluted with HCl in methanol, the solvent is evaporated, and the acids are methylated with diazomethane.

Table 1.6 EXAMPLE CLASSES AND ANALYTES RECOVERED BY BATCH L-L PARTITION PROTOCOL AT pH 1.0 (ESSA)

- B.P. ~200°C up to limit of chromatography (methyl esters)
- $pK_a < 8.0$

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)[a]
Carboxylic Acids	valeric	82 ± 3 (4)
	benzoic	92 ± 5 (6)
	dichloroacetic	73 ± 7 (10)
	p-toluic	91 ± 6 (6)
	n-octanoic	82 ± 8 (9)
	o-methoxybenzoic	90 ± 6 (7)
	o-chlorobenzoic	91 ± 2 (2)
	n-nonanoic	85 ± 7 (9)
	p-nitrobenzoic	88 ± 8 (9)
	2-naphthoic	94 ± 8 (9)
	n-decanoic	87 ± 5 (6)
	lauric	106 ± 12 (11)
	2,4-dichlorophenoxyacetic	110 ± 10 (9)
	palmitic	89 ± 10 (11)
	oleic	63 ± 11 (17)
	p-*t*-butylbenzoic	96 ± 4 (5)
	myristic	90 ± 18 (20)
Phenols	2-nitro-*p*-cresol	88 ± 8 (9)
	2,4-dichlorophenol	88 ± 4 (5)
	2,3,6-trichlorophenol	93 ± 5 (5)
	pentachlorophenol	100 ± 19 (19)
	2,4,5-trichlorophenol	99 ± 4 (4)
Deuterated Internal Standards	d_{13}-heptanoic acid	65 ± 8 (12)
	d_5-benzoic acid	92 ± 5 (6)

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[a] Recovery is a mean value for triplicate determinations in drinking water, spiked at 50-100 ppb (nominally 55 ppb). S.D. = standard deviation, C.V. = coefficient of variation. Recoveries were not determined in more complex waters.

Table 1.7. EXAMPLE CLASSES AND ANALYTES RECOVERED BY ANION-EXCHANGE/DISTILLATION ANALYTICAL PROTOCOL (VOSA)

- Boiling point range ~100°C-250°C (free acids)
- $pK_a < 6.0$

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)	Overall Recoveries[a] Mean ± S.D. (C.V.)	Relative Retention (C.V.)[b] 2-Fluorobiphenyl	Relative Retention (C.V.)[b] d_7-Butyric Acid, Benzyl Ester
Volatile Carboxylic Acids	acetic	-	-[c]	0.623 (0.23)	0.648 (0.02)
	acrylic	119 ± 8 (7)	90 ± 25 (28)	0.786 (0.11)	0.817 (0.09)
	n-propanoic	101 ± 5 (5)	-	0.806 (0.09)	0.839 (0.10)
	isobutyric	-	42 ± 29 (69)	0.886 (0.05)	0.922 (0.08)
	methacrylic	80 ± 6 (7)	57 ± 19 (34)	0.947 (0.04)	0.986 (0.07)
	n-butyric	-	57 ± 8 (14)	0.970 (0.05)	1.010 (0.07)
	2-methylbutyric	93 ± 9 (10)	74 ± 17 (23)	1.031 (0.10)	1.066 (0.10)
	isovaleric	105 ± 11 (10)	88 ± 15 (17)	1.040 (0.14)	1.075 (0.14)
	crotonic	102 ± 10 (8)	77 ± 22 (28)	1.069 (0.05)	1.113 (0.06)
	n-valeric	88 ± 10 (11)	75 ± 12 (16)	1.146 (0.04)	1.192 (0.07)
	2-methyl-2-butenoic	-	-	1.161 (0.23)	1.120 (0.26)
	2-methylcyclopropane-carboxylic	92 ± 6 (6)	74 ± 17 (24)	1.122 (0.22)	1.160 (0.32)
	3,3-dimethylacrylic	91 ± 9 (10)	72 ± 17 (23)	1.151 (0.24)	1.190 (0.28)
	2-ethylbutyric	67 ± 11 (16)	57 ± 9 (15)	1.184 (0.06)	1.231 (0.05)
	3-hexenoic	70 ± 7 (10)	61 ± 9 (14)	1.220 (0.27)	1.261 (0.30)
	2-hexenoic	62 ± 10 (16)	61 ± 3 (4)	1.285 (0.33)	1.328 (0.34)
	n-hexanoic	70 ± 7 (10)	60 ± 9 (16)	1.317 (1.0)	1.370 (1.0)
	cyclopentylacetic	54 ± 7 (13)	47 ± 6 (13)	1.379 (0.34)	1.427 (0.55)
	n-heptanoic	62 ± 10 (16)	48 ± 13 (27)	1.473 (0.10)	1.533 (0.09)
	cyclohexane carboxylic	32 ± 2 (6)	32 ± 2 (6)	1.557 (0.09)	1.619 (0.09)
	n-octanoic	54 ± 7 (13)	46 ± 7 (15)	1.626 (0.09)	1.692 (0.10)
	cyclohexylacetic	39 ± 6 (15)	37 ± 3 (9)	1.690 (0.10)	1.757 (0.10)
	n-nonanoic	-	-	1.773 (0.12)	1.845 (0.10)
	Tiglic acid	77 ± 4 (5)	77 ± 4 (5)[d]		
Deuterated Internal Standard	d_7-butyric acid	93 ± 3 (3)	85 ± 12 (14)	0.961 (0.03)	1.00

[a] Recovery is mean value for triplicate determinations from drinking water, spiked at 0.3 ppb, and for triplicate determinations from a 60/40 industrial/municipal wastewater, spiked at 120 ppb. S.D. = standard deviation, C.V. = coefficient of variation.

[b] Relative retention, as their benzyl ester derivatives, to 2-fluorobiphenyl and d_7-butyric acid, benzyl ester. DB-1 fused silica capillary column, 30 M x 0.34 mm x 1.0 μ film thickness.

[c] Not determined.

[d] In drinking water only.

Table 1.8. EXAMPLE CLASSES AND ANALYTES RECOVERED BY ANION-EXCHANGE ANALYTICAL PROTOCOL (NOVA)

- Boiling point range ~250°C to limit of chromatography (methyl esters)
- $pK_a < 6.0$

Applicable Chemical Classes	Example Analytes	Deionized Water Recoveries Average ± Range	Overall Recoveries Mean ± S.D. (C.V.)[a]
Carboxylic Acids	malonic	78[b]	78[c]
	succinic	106±0	87 ± 40 (45)[d]
	azelaic	73±0	64 ± 19 (30)[e]
	m-nitrobenzoic	-[f]	68 ± 5 (7)[g]
	terephthalic	-	55 ± 1 (2)[g]
	3,4-dichlorobenzoic	-	42 ± 4 (9)[g]
	2,4,5-trichlorophenoxyacetic	-	67 ± 8 (13)[h]
	oleic	-	9 ± 2 (22)[g]
Sulfonic Acids	methanesulfonic	115 ± 6 (5)	115 ± 6 (5)[c]
	benzenesulfonic	130	84 ± 38 (45)[g]
	p-toluenesulfonic	74	98 ± 23 (24)[h]
	4-chlorobenzenesulfonic	53	92 ± 6 (7)[g]
	2-naphthalenesulfonic	-	110 ± 15 (14)[g]
Miscellaneous	benzenephosphoric		104 ± 50 (50)[i]
	benzenephosphonic	144	140 ± 20 (14)[g]
	pentachlorophenol	-	62 ± 7 (11)[g]

(continued)

Table 1.8 (cont'd.)

Applicable Chemical Classes	Example Analytes	Deionized Water Recoveries Average ± Range	Overall Recoveries Mean ± S.D. (C.V.)
Deuterated Internal Standard	2-naphthalenesulfonic acid-$d_7 \cdot H_2O$	-	110 ± 15 (14)[g]

[a] All recoveries at nominal 50 ppb; S.D. = standard deviation, C.V. = coefficient of variation.

[b] Single determination.

[c] Recovery from deionized water only.

[d] Recovery from deionized water and one municipal and three industrial effluents.

[e] Recovery from deionized water and one municipal and two industrial effluents.

[f] Not determined.

[g] Recovery from one industrial effluent.

[h] Recovery from one municipal and two industrial effluents.

[i] Recovery from two industrial effluents.

Strong amines (SAM), such as hexylamine, dibutylamine, and 2,6-dimethylpiperidine (Table 1.9) are isolated from the water sample on Biorad AG 50W-X8 cation exchange resin, then eluted with sodium hydroxide in acetonitrile: water solution. The eluate is acidified (HCL), the solution is evaporated to dryness, and the amine hydrochloride salts are dissolved in base and extracted with methyl-t-butyl ether. The extract is split, half is derivatized with pentafluorobenzyl bromide to make the pentylfluorobenzyl tertiary amines from the secondary amines (SAM-S), and half is derivatized with pentafluorobenzaldehyde to make Schiff bases of the primary amines (SAM-PT).

Tertiary amines are also separated by this protocol and quantified (underivatized) in the primary amine fraction. Certain weak bases (e.g., anilines) may be detected in these fractions, but are measured in the pH 8 extractable fraction (WABN), where they are extracted more efficiently. (Quaternary amines are not addressed by the MAS.)

1.2.4 Extract Processing

Extractable and ionic fractions require further processing before GC/MS. The necessary derivatization steps, for example, are described above and summarized in Figure 1.1.

The pH 8 extractable fraction (WABN-BL) of many industrial effluents will require clean-up and sub-fractionation before effective separation can be achieved, even on capillary column. First, however, a scouting procedure is implemented to determine whether clean-up is necessary. The crude extract is analyzed by GC using a packed column and flame ionization detection; baseline rise relative to a separately run standard is the evaluation criterion. Clean-up, if necessary, is on a silica gel column from which three fractions (WABN-BL1, -BL2, and -BL3) are eluted using pentane, methylene chloride, methanol, and their mixtures.

Concentration of extracts for GC/MS analysis is by Kuderna-Danish evaporation down to 4 mL, followed by nitrogen blowdown to 0.5 mL or 1.0 mL using a modified Snyder column. "External" standards (Table 1.2) are added to each final extract just before GC/MS analysis to monitor recovery of the deuterated internal standards that were added to the original water samples. The external standard for the purgeable fractions

Table 1.9. EXAMPLE CLASSES AND ANALYTES RECOVERED BY CATION-EXCHANGE ANALYTICAL PROTOCOL (SAM)

- Boiling point range ~30 to 300°C (free amines)
- $pK_a > 6$

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)[a]	Overall Recoveries Mean ± S.D. (C.V.)[a]
Primary and Tertiary Amines (SAM-PT)	allylamine	80 ± 8 (10)	86 ± 26 (31)[b]
	n-butylamine	-	75 ± 11 (15)[c]
	*i*sobutylamine	-	76 ± 18 (24)[c]
	sec-butylamine	-	77 ± 19 (25)[c]
	t-butylamine	101 ± 7 (7)	58 ± 24 (41)[b]
	n-hexylamine	45 ± 9 (20)	58 ± 16 (27)[b]
	cyclohexylamine	70 ± 1 (1)	76 ± 10 (13)[b]
	2-ethylhexylamine	-	75 ± 10 (13)[c]
	benzylamine	60 ± 0 (0)	66 ± 8 (12)[b]
	1,2-ethane diamine	-	73 ± 20 (27)[c]
	tri-*n*-butylamine	-	75 ± 25 (33)[c]
Secondary Amines (SAM-S)	diallylamine	136 ± 34 (25)	98 ± 19 (20)[b]
	di-*n*-propylamine	108 ± 45 (42)	70 ± 18 (26)[b]
	piperidine	21 ± 9 (43)	22 ± 12 (55)[b]
	morpholine	144 ± 12 (9)	32 ± 49 (155)[b]
	2-methylpiperidine	118 ± 113 (11)	56 ± 30 (53)[b]
	di-*n*-butylamine	112 ± 9 (8)	56 ± 23 (42)[b]
	2,6-dimethylpiperidine	75 ± 12 (16)	57 ± 25 (43)[b]
	dicyclohexylamine	46 ± 15 (23)	40 ± 12 (31)[b]

(continued)

Table 1.9 (cont'd.)

Applicable Chemical Classes	Example Analytes	Drinking Water Recoveries Mean ± S.D. (C.V.)	Overall Recoveries Mean ± S.C. (C.V.)
Internal Standards	d_9-butylamine	75 ± 27 (36)	75 ± 20 (27)[b]
	d_4-phenylethylamine	-	
	N-ethyl-2-fluorobenzylamine	-	

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[a] Recovery for triplicate determination; S.D. = standard deviation, C.V. = coefficient of variation.

[b] Recovery from drinking water, spiked at 35 ppb, and two municipal and three industrial effluents spiked at 110 ppb.

[c] Recovery from two municipal and three industrial effluents spiked at 110 ppb.

(VO and NEWS) is perfluorotoluene. External standards for all the other fractions are 2-fluorobiphenyl and/or 4-fluoro-2-iodotoluene.

1.2.5 Gas Chromatography

As shown in Figure 1.1, as many as 10 extracts or fractions may be obtained from one sample if the entire MAS protocol is applied (this may be reduced to 7 if cleanup of the pH 8 (WABN) extract is not necessary, and if the primary and secondary amine fractions can be mixed for a single GC/MS analysis). Glass or fused silica capillaries are prescribed. Bonded phase (e.g., Durabond DB-1 or DB-5), wide-bore, thick film (1.0 μ), 30 or 60 M fused silica columns are recommended for inertness, stability, and sample capacity. In all cases performance standards (see Quality Assurance) rather than specific columns are specified (see Quality Assurance) in the analytical protocol. No more than four different GC columns should be necessary for the entire MAS. The analytical protocol for each fraction prescribes optimum GC conditions.

1.2.6 Qualitative Analysis

Sample components are identified by established GC/MS/COMP techniques. No research was conducted on the MAS identification procedures. GC/MS data are stored on tape or disk. Internal standards in each extract are used as reference points for retention time measurements as well as for quantification. Compounds are identified by computer searching of mass spectra data banks or by manual interpretation.

1.2.7 Quantitative Analysis

Extensive recovery studies were conducted during development of the MAS (Tables 1.3-1.9). Approximately 280 model compounds from a wide variety of chemical classes and physical property groups were dosed into representative samples of several major types of water (distilled and drinking water, and municipal and/or industrial effluents). Recoveries were determined and average recovery factors are stored in a computer data bank. Relative molar response (RMR) factors (relative to the deuterated internal standards), based on MS selected ion peak areas, were also determined and stored in the data bank. (Appendix B is a hard copy of these data.) The MAS user can use these data banks and a computer program developed for the MAS (MASQUANT, Appendix C) to calculate the concentration in the original water sample of these model compounds as they are identified. For compounds that are not in the data bank, concentrations can be estimated by using RMR and recovery factors for structurally similar compounds in the data bank. (See summary tables in Section 1.4.)

During development of the MAS, it was determined that GC/MS data should be collected in the full scan mode rather than by selective ion monitoring. It was also shown, however, that quantification should be based on peak areas (or height) of selected ions of compounds rather than on the total ion current of that compound. Thus, RMR factors involve ratios of ion peak areas and moles of the model compound to ion areas and moles of the internal standard selected for quantification (usually the closest eluting standard). These ion areas depend strongly on the GC/MS instrumentation and MS tuning. Thus, it is necessary for each MAS user to either develop his own RMR values or alternatively measure a few compound RMRs at the MS tune specified and use a linear regression plot of these against tabulated RMRs of the same compounds to develop a correction factor (slope) for the tabulated RMRs. Tabulated RMR values are those in the computer data bank and are provided as part of the MAS protocols (Appendix B).

RMR values developed by the linear regression correction procedure will not be as accurate as those measured directly by the user. The user can measure his own RMR factors using one or more solutions of the model compounds within each protocol class (e.g., VO, NEWS, ESSA, etc.) containing all of the appropriate internal standards for that class. For example, development of RMRs for the MAS computer data bank for MASQUANT required analysis of about 15 solutions containing up to 35 compounds in each. These solutions were analyzed in replicate (usually six runs) on a Finnigan 4021 GC-MS-COMP system.

Even if the user develops his own RMR data bank, it is necessary for him to track the tune of his mass spectrometer daily by chromatographing a system performance mixture prescribed in each analytical protocol. If the tune is significantly different from that used when his RMR values were measured, the same linear regression procedure can be used to correct the tabulated RMRs for the current tune. The linear regression and correction can be done by MASQUANT.

To use MASQUANT for quantification, the user must provide sample component identities, some information as to the specific MAS analytical steps used, selected ion peak areas for each sample component and the appropriate (nearest) internal standard, and the concentration of the internal standard in the original water sample. If the identified sample component is one of the model compounds, the computation program will select appropriate RMR and recovery factors for that component and calculate its concentration in the original sample. Complete instructions for MAS quantification are given in Chapter 13 and Appendices B and C.

As mentioned above, if the identified sample component is not a model compound, RMR and recovery factor of a structurally similar compound in the data bank may be used for an estimate of concentration (see summary tables in Section 1.4). In addition to this obvious source of error, an additional error is involved in using any recovery factors from the data bank. Since sample matrices used for recovery studies were only representative of the various water types, and since all recovery values for all matrices studied were averaged to give the factor in the data bank, errors will occur in applying these factors to other samples and the error is dependent on the matrix differences between the sample being analyzed and the representative recovery samples.

Footnotes on Tables 1.3-1.9 give more information on sample matrices used for recovery studies. Separate recovery values are given for drinking water for all MAS fractions except nonvolatile acids. These are more accurate than MASQUANT data for drinking water analysis since the MASQUANT (Appendix B) data bank values are averages over all water types studied. On the other hand, recoveries using the WABN-FU protocols (for industrial effluents) are so matrix dependent that there is no representative sample matrix for recovery studies (see Footnote a, Table 1.5). The user must generate his own recoveries for his own sample matrix.

1.2.8 Quality Assurance/Quality Control

As discussed above, extensive recovery studies with model compounds were performed during development of each MAS procedure. Statistical analysis techniques were applied to replicate recovery determinations to arrive at the precision associated with each compound's recovery. The same statistical approach was used in measuring compound RMRs. The sum of the tabulated recovery precision and RMR precision values for an analyte gives the best precision to be expected with use of the appropriate MAS protocol. This should be considered when expressing the quality of data generated by application of the MAS. If the user generates his own RMR and/or recovery data, the precision and accuracy will be different from the tabulated values since they will include terms for user operational errors, different levels of GC/MS/COMP instrument precision, and new matrix effects.

Of most importance are the quality control steps prescribed for the user in each protocol. Some of these steps are outlined below.

1.2.8.1 System Performance Solution--

One important quality assurance step is the use of standard performance solutions to check performance of the GC/MS/COMP each day. The MAS prescribes a performance standard solution and corresponding criteria of acceptance for each analytical protocol. Solutions include compounds to measure GC peak asymmetry, separation number, resolution, polarity, and column acidity and basicity; capacity of the capillary column; inertness of the GC to MS transfer line; limits of detection of the MS/COMP system; and tune of the MS. These solutions also contain deuterated internal standards and the external standard appropriate to each protocol for RMR verification and periodic determination of the RMR correction factor by linear regression, if necessary.

1.2.8.2 Internal and External Standards--

Comparison of the recovered quantity of deuterated internal standards to the quantity of external standard added to the extract just before GC/MS analysis reveals recovery deficiencies, thus serving as a check to indicate malfunctions of the MAS analytical procedure. The primary use of deuterated internal standards is for quantification; reference to an internal standard is generally accepted as the most accurate quantitation technique available for the GC/MS analysis of organics in water. Internal standards may also be used as retention time indices for an aid in compound identification.

1.2.8.3 Sample Scouting--

As described earlier, several sample scouting measurements are prescribed to characterize water quality and in turn allow selection of the appropriate and optimal analytical techniques for a particular water sample. Scouting measurements also help in determining optimum sample size and dilution factor for certain protocols.

1.2.8.4 Blanks, Controls, Duplicates, and Surrogate Samples--

Requirements and procedures for field and laboratory blanks, spiked field and laboratory controls, and duplicate and surrogate samples are specified in each sampling protocol. Procedures for cleaning glassware and apparatus and other steps to assure quality of measurement are also specified throughout the MAS.

1.2.8.5 MAS Test Samples--

For each MAS protocol, instructions are given for preparing controls (for quality assurance) by dosing known amounts of analytes into reagent water. Test samples for practicing and checking MAS procedures may be prepared in the same way.

1.3 TIME REQUIREMENTS FOR THE MAS

A very preliminary estimate of time per comprehensive MAS sample, or a corresponding quality assurance sample, is 80 hours. This is for a laboratory analyzing only a few, say 10 to 50, samples per year, using personnel who are experienced in trace organic analysis of water and set up with the equipment and techniques used for the MAS.

It should be remembered that the MAS protocols were developed and designed as separate entities so that a laboratory could analyze only the fractions appropriate to its mission. The cost for analyzing pH 8 extractables, for example, might be only 10% of that for a comprehensive MAS analysis.

1.4 SUMMARY OF RECOVERY AND PRECISION FOR MAS PROTOCOLS

Tables 1.10-1.12 summarize the recovery data reported in Tables 1.3-1.9 for the chemical classes corresponding to each MAS protocol. Footnotes to Table 1.10 give information on sample matrices and spiking levels used for recovery studies. Several observations can be made regarding these data (Table 1.11): 1) recoveries for volatile (purgeable) organics are highest (these purgeables data are for a wide variety of compound classes from several types of water); 2) neutral water soluble organics, a new class of organic analytes, are recovered adequately with adequate precision; and

Table 1.10. SUMMARY OF ALL MAS RECOVERY DATA (INCLUDING DEUTERATED INTERNAL STANDARDS)

PROTOCOL CLASS Chemical Class (Examples)	Compounds Studied	Recovery Range %	Mean Recovery, %	CV Range, %	Mean CV, %	Footnotes
VOLATILE (PURGEABLE) ORGANICS (VO)						
Aromatic Hydrocarbons (benzene; naphthalene)	9	59-113	85	3-35	11	a
Halogenated Aromatics (chlorobenzene; 1,2,3-trichlorobenzene)	7	91-106	100	2-30	15	a
Misc. Aromatic Compounds (anisole)	1	-	68	-	12	a
Aliphatic and Alicyclic Hydrocarbons (cyclohexane; *n*-tridecane)	11	44-120	82	1-40	16	a
Halogenated Aliphatic Hydrocarbons (chloroform; 1,4-dibromobutane)	7	77-118	90	4-16	9	a
Miscellaneous Oxygen & Sulfur Compounds (diethyl ether; hexyl ether)	5	70-115	93	3-26	11	a
Deuterated Standards (d_5-bromoethane; 2,4,6-d^3-anisole; d_5-chlorobenzene; d_8-naphthalene)	4	57-120	90	10-25	18	a
NEUTRAL WATER SOLUBLE ORGANICS (NEWS)						
Alcohols (1-propanol; 1-heptanol)	4	106-131	115	18-25	22	b
Aldehydes (*n*-butyraldehyde; crotonaldehyde)	3	72-83	79	25-32	29	b
Esters (methyl formate; ethyl butyrate)	7	45-93	74	4-17	7	c
Ethers (tetrahydrofuran; dioxane)	2	50-79	65	14-31	23	b
Ketones (methyl ethyl ketone; cyclohexanone)	2	65-69	67	2-32	17	b
Nitriles (acrylonitrile; benzonitrile)	4	57-95	83	6-14	9	b
Nitro Compounds (nitromethane; nitrobenzene)	3	70-96	86	5-13	9	b
Deuterated Standards (d_9-*t*-butanol; d_5-nitrobenzene)	2	93-98	96	7-10	9	b
WEAK ACIDS, BASES, AND NEUTRALS (WABN-SC and WABN-BL)						
Weak Acids (phenol; 2,4-dichlorophenol)						
accumulator column	6	55-95	77	5-23	11	d
batch liquid-liquid	12	49-118	71	5-27	13	e
Weak Bases (aniline; carbazole)						
accumulator column	16	53-95	82	0-16	7	d
batch liquid-liquid	15	40-86	64	6-40	24	e
Alkanes (*n*-decane; *n*-tridecane)						
accumulator column	8	42-66	52	6-20	15	d
batch liquid-liquid	11	45-82	62	13-31	21	f
Aliphatic Ketones, Alcohols, and Esters (fenchone; methyl stearate)						
accumulator column	7	49-94	75	1-22	10	d
batch liquid-liquid	9	49-111	71	10-43	25	e

(continued)

Table 1.10 (cont'd.)

PROTOCOL CLASS Chemical Class (Examples)	Compounds Studied	Recovery Range %	Mean Recovery, %	CV Range, %	Mean CV, %	Footnotes
Misc. Aliphatic Compounds (di-*t*-butyldisulfide; tributylphosphate)						
accumulator column	6	40-92	72	2-36	18	d
batch liquid-liquid	4	57-104	75	9-36	21	e
Aromatic Hydrocarbons (2-methylnaphthalene, pyrene)						
accumulator column	10	60-87	74	1-27	16	d
batch liquid-liquid	7	48-118	79	13-41	24	e
Halogenated Aromatics (*o*-chloroanisole; hexachlorobenzene)						
accumulator column	10	56-100	73	1-42	16	d
batch liquid-liquid	9	43-107	68	13-33	20	e
Aromatic Aldehydes and Ketones (*o*-tolualdehyde; acetophenone)						
accumulator column	3	88-96	92	2-17	12	d
batch liquid-liquid	4	43-105	69	6-19	13	e
Aromatic Esters and Sulfonates (benzylacetate, ethyl-*p*-toluenesulfonate)						
accumulator column	6	46-87	69	7-17	12	d
batch liquid-liquid	7	55-138	84	3-19	11	e
Misc. Aromatic Compounds (nitrobenzene; tetraphenyltin)						
accumulator column	6	47-89	74	6-24	13	d
batch liquid-liquid	10	48-105	68	8-33	17	e
Deuterated Standards (d_{10}-xylene; d_5-phenol; d_5-acetophenone; d_5-phenylethanol; d_5-nitrobenzene; d_5-propiophenone; d_8-naphthalene; d_9-acridine; d_{12}-perylene)						
accumulator column	9	55-93	78	4-21	10	d
batch liquid-liquid	8	40-78	58	11-40	26	e
EXTRACTABLE SEMIVOLATILE STRONG ACIDS (ESSA)						
Carboxylic Acids (benzoic acid; palmitic acid)	17	63-110	89	2-20	9	g
Phenols (2-nitro-*p*-cresol; pentachlorophenol)	5	88-100	94	4-19	8	g
Deuterated Standards (d_{13}-heptanoic acid; d_5-benzoic acid)	2	65-92	79	6-12	9	g
VOLATILE STRONG ACIDS (VOSA)						
Volatile Carboxylic Acids (acrylic acid; *n*-octanoic acid)	16	46-90	65	4-34	19	h
Deuterated Standards (d_7-butyric acid)	1	-	85	-	14	h
NONVOLATILE ACIDS (NOVA)						
Carboxylic Acids (succinic acid; 2,4,5-trichlorophenoxyacetic acid)	6	42-87	64	2-45	18	i
Sulfonic Acids (benzenesulfonic acid; 2-naphthalenesulfonic acid)	4	84-110	96	7-45	23	i
Misc. Nonvolatile Acids (benzenephosphoric acid; pentachlorophenol)	3	62-140	102	11-50	25	i
Deuterated Standards (2-naphthalenesulfonic acid-$d_7 \cdot H_2O$)	1	-	110	-	14	i

(continued)

Table 1.10 (cont'd.)

PROTOCOL CLASS Chemical Class (Examples)	Compounds Studied	Recovery Range %	Mean Recovery, %	CV Range, %	Mean CV, %	Footnotes
STRONG AMINES (SAM-PT and SAS-S)						
Primary and Tertiary Amines (*n*-butylamine; tri-*n*-butylamine)	11	58-86	72	12-41	24	j
Secondary Amines (diallylamine; 2-methylpiperidine)	6	40-98	63	20-53	36	j
Deuterated Standards (d_9-butylamine; d_4-phenylethylamine; N-ethyl-2-fluorobenzylamine)	1	-	75	-	27	j,k

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[a] Mean recoveries are for triplicate determinations from drinking water, spiked at 0.2 to 1.8 ppb (nominally 1 ppb), plus triplicate determinations from a 60/40 industrial/municipal wastewater, spiked at 30 to 87 ppb (nominally 50 ppb).

[b] Mean recoveries are for triplicate determinations from drinking water, spiked at 0.8 to 1.2 ppb (nominally 1 ppb), plus triplicate determinations from a 60/40 industrial/municipal wastewater, spiked at 40 to 63 ppb (nominally 50 ppb).

[c] Mean recoveries are for triplicate determinations from 60/40 industrial/municipal wastewater only, spiked at 40 to 63 ppb (nominally 50 ppb).

[d] Mean recoveries are for triplicate determinations from drinking water, spiked at 0.5 to 5 ppb (nominally 1 ppb), using XAD-4 resin sorbent columns.

[e] Mean recoveries are for triplicate determinations from a 60/40 industrial/municipal wastewater, or, for about 1/4 of the total compounds, from reagent water spiked at 15 to 50 ppb (nominally 25 ppb), using batch liquid-liquid extraction, with clean-up included.

[f] Mean recoveries are for triplicate determinations from reagent water only, with clean-up steps included. (Interferences prevented recovery determinations from wastewater).

[g] Mean recoveries are from triplicate determinations from drinking water only, spiked at 50-100 ppb (nominally 55 ppb). Recoveries were not determined from more complex waters.

[h] Mean recoveries are for triplicate determinations from drinking water, spiked at 0.3 ppb, plus triplicate determinations from a 60/40 industrial/municipal wastewater, spiked at 120 ppb.

[i] Mean recoveries are for triplicate determinations from several industrial and municipal effluents.

[j] Mean recoveries are for triplicate determinations from three industrial and two municipal effluents spiked at 110 ppb, and including, in some cases, triplicate determinations from drinking water spiked at 35 ppb.

[k] Recoveries determined for only one (d_9-butylamine) of the three internal standards.

Table 1.11. SUMMARY OF ALL MAS RECOVERY DATA BY PROTOCOL CLASS

Protocol Class	No. Compounds	Mean Recovery, %	Mean CV, %
Volatile (purgeables) Organics (VO)	44	89	13
Neutral Water Soluble Organocs (NEWS)	27	84	14
Weak Acids, Bases, and Neutrals (WABN)			
accumulator column (WABN-SC)	87[a,b]	74[b]	12[b]
batch liquid-liquid (WABN-BL)	96[a]	69	20
Extractable Semivolatile Strong Acids (ESSA)	24[b]	89[b]	9[b]
Volatile Strong Acids (VOSA)	17	66	19
Nonvolatile Acids (NOVA)	14[c]	85[c]	20[c]
Strong Amines (SAM-PT and SAM-S)	18	69	28
	327[a]	76[d]	16[d]

[a] Sixty-nine compounds were used for both accumulator column and batch liquid-liquid recovery studies; the total number of different compounds in this table is therefore 258.

[b] ESSA and WABN-SC recovery data are for drinking water only.

[c] NOVA recovery data are for industrial and municipal effluents only.

[d] Mean recoveries and mean CVs were calculated from values for the 327 individual compounds.

Table 1.12. SUMMARY OF MAS RECOVERY DATA FOR ORGANICS IN DRINKING WATER BY PROTOCOL CLASS[a]

Protocol Class	No. Compounds	Spiking Range (ppb)[b]	Nominal Spiking Level (ppb)[b]	Mean Recovery %	Mean CV %
Volatile (Purgeable) Organics (VO)	52	0.2-1.8	1	90	10
Neutral Water Soluble Organics (NEWS)	25	0.8-1.2	1	84	16
Weak Acids, Bases, and Neutrals (WABN, accumulator column)	87	0.5-5	1	74	12
Extractable Semivolatile Strong Acids (ESSA)	24	50-100	55	89	9
Volatile Strong Acids (VOSA)	18	-	0.3	82	10
Strong Amines (SAM-PT and SAM-S)	11	-	35	81	16
	217			82[c]	12[c]

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[a] For triplicate determinations from drinking water. Nonvolatile Acids (NOVA) were not determined in drinking water.

[b] Level spiked into water sample.

[c] Mean recoveries and mean CVs were calculated from values for the 217 individual compounds.

3) two classes of ionic compounds, volatile strong acids and strong amines, are characterized by relatively low recoveries and poor precision. It is also seen from Table 1.11 that recovery of organics using accumulator columns is better than with batch liquid-liquid extraction in a separatory funnel, and that precision is also better. Matrix effects may be more important than the extraction technique, however; only drinking water was extracted by accumulator column, whereas more complex matrices were extracted by the batch technique.

Table 1.12 shows summarized recovery data for organics in drinking water only, by MAS protocol. (These data were integrated into the total recovery data of Tables 1.10 and 1.11.) Spiking ranges and nominal spiking levels are significantly lower than those used for recoveries from industrial and municipal effluents (see footnotes to Table 1.10. Recoveries for VO and NEWS compounds are practically the same as those given in Table 1.11; i.e., matrix effects or spiking levels did not make a significant difference in the summarized data. Extractable semivolatile strong acids were recovered well from drinking water, with good precision, but the spiking level was relatively high. The other ionic classes of organics (VOSA and SAM) were recovered at significantly higher levels and with better precision from drinking water than from other matrices (cf. Table 1.11).

Mean recoveries over all protocols for water types studied (Table 1.11) for 327 determinations (258 different compounds) was 76% with a mean relative standard deviation (for 3 or more measurements) of 16%. For drinking water (Table 1.12), the mean recovery for 217 spiked compounds was 82%, with a mean RSD of 12%.

CHAPTER 2

SAMPLING AND SAMPLE HANDLING FOR VOLATILE ORGANICS (VO)

2.1 INTRODUCTION

This section describes the collection and handling of drinking water, surface water, and municipal and industrial wastewater effluent samples for the analysis of volatile (purgeable) organics (VO). For all waters, a 12 mL and a 250 mL water sample is collected for scouting measurements and analysis, respectively. Immediately after collection and scouting, residual chlorine in drinking water and treated municipal water will be quenched using sodium thiosulfate. Samples are collected in containers with no headspace, capped with crimp-top, Teflon-lined septum caps, and the bottles inverted for shipment and storage. Upon arrival at the laboratory, internal standards are released into the samples, which are then stored at 4°C until analysis.

Table 2.1 illustrates the sampling regime with quality control and assurance samples for up to 20 different field samples. Duplicates, surrogates, field and laboratory controls, and field and laboratory blanks are prescribed. The exact number is dependent upon the number of field samples to be collected. For example, if 11 field samples are to be collected, then two duplicates (a duplicate of each of two field samples), one surrogate, and one each of field and laboratory controls and blanks are incorporated as part of the QC/QA. Thus, a total of 18 analyses would be performed for 11 field samples (Table 2.1).

Control samples will be prepared by adding known quantities of representative target compounds to reagent (free of VO compounds) water. Blanks are unspiked reagent water. Laboratory controls and blanks, after preparation, are stored at 4°C in the laboratory until analysis. Field controls and blanks are shipped to the sampling site and handled in the same manner as field samples in terms of cooling, shipping and storing.

Table 2.1. NUMBER OF QUALITY CONTROL SAMPLES ASSOCIATED WITH FIELD SAMPLES

No. of Samples to be Collected	Duplicates	Surrogates	Controls		Blanks		Total No. of Analyses
			Field	Laboratory	Field	Laboratory	
1	1	-	-	-	1	1	4
2	1	-	-	-	1	1	5
3	1	-	-	-	1	1	6
4	1	-	1	-	1	1	8
5	1	-	1	-	1	1	9
6	1	-	1	-	1	1	10
7	1	-	1	-	1	1	11
8	1	-	1	-	1	1	12
9	1	-	1	-	1	1	13
10	1	-	1	-	1	1	14
11	2	1	1	1	1	1	18
12	2	1	1	1	1	1	19
13	2	1	1	1	1	1	20
14	2	1	1	1	1	1	21
15	2	2	1	1	1	1	23
16	2	2	1	1	1	1	24
17	2	2	1	1	1	1	25
18	2	2	1	1	1	1	26
19	2	2	1	2	2	2	30
20	2	2	2	2	2	2	32

Upon the return to the laboratory, representative VO target compounds are spiked into replicate field samples (the number of samples treated in this manner is specified in Table 2.1) for use as surrogate samples. Comparison of the analytical results for controls, blanks and duplicates to results for field samples will provide a measure of uncontrolled contamination and/or analyte losses during sample collection, shipment, and storage, the homogeneity of the sample matrix and the accuracy and precision associated with sample preparation and analysis. Recovery of internal standards and spiked compounds from the surrogate samples will provide information on matrix effects on method accuracy and precision (i.e., if there are two or more surrogates).

If more than 20 samples are to be collected, the additional field samples are matched with the prescribed number of duplicates, surrogates, etc. For example, if 33 samples are to be collected, then lines for 20 and 13 samples, respectively, are consulted and the number of duplicates, surrogates, etc., are a sum of these two.

2.2 MATERIALS AND REAGENTS

The following materials are required for collecting a set of 20 water samples plus two duplicate and two surrogate samples and two each of field and laboratory controls, and two each of field and laboratory blanks for the analysis of purgeable volatile organics (VO).

(1) Thirty-two, narrow mouth, 243 mL amber glass bottles with Teflon lined septum screw cap or (crimpable) caps. One 3 dram (12 mL), septum-capped vial. Thirty-two Teflon coated magnetic stirring bars; one for each bottle.

(2) One Bausch and Lomb Mini Spec 20 portable spectrophotometer.

(3) One 25 mL mixing cylinder (Hach Chlorine Determination kit).

(4) Materials (e.g., "Blue Ice®") for chilling the sample during shipment and storage; insulated shipping containers for samples.

(5) 25 mL volumetric flasks for preparing standard solutions; 2 mL glass ampules.

(6) 10 μL GC-type microliter syringe, Teflon tipped plunger.

(7) Reagents:

(a) reagent water - reagent water is defined as water in which no interferences are observed at the detection limit for the parameters of interest. Two liters of water from a purification system (Millipore Super-Q® or equivalent), purged at 80°C for 30 min with cryogenically (LN_2) cleaned He gas at 15 mL/min may be used.

(b) Thirty-two microcapsules containing internal standards (NBS prepared) as shown in Table 2.2.

(c) 25 mL standard solution (Table 2.3). Purgeables standard solutions are prepared from pure, neat reagent materials.

(d) 250 mL $Na_2S_2O_3$ solution - 0.05 $\underline{M}$ in reagent water.

(e) DPD Total Chlorine Reagent Powder Pillow (Hach Chemical Co.).

2.3 PREPARATION OF STANDARD SOLUTIONS

2.3.1 Internal Standards

These microcapsules are used as received from EPA (or NBS, Table 2.2).

2.3.2 Standard Solution of Purgeable Compounds

(1) Prepare a standard solution by accurately measuring 3 µL of neat liquid standard (Table 2.3) using a 10 µL syringe into the expanded area of a 25 mL volumetric flask containing about 25 mL

Table 2.2. NBS MICROCAPSULES CONTAINING PURGEABLE INTERNAL STANDARDS IN METHANOL[a]

Internal Standard	µg/L[b] Set No. 1	µg/L[b] Set No. 3
d_5-bromoethane	0.56	6.6
2,4,6-d_3-anisole	4.2	25.6
d_5-chlorobenzene	7.2	5.4
d_8-naphthalene	5.8	48.7

[a] Concentrations are based upon the production run made by NBS. This production run is the "400" series.

[b] Concentration after dosing in 250 mL of water. Set No. 2 was an experimental set and is not prescribed in the MAS and, thus, it is not listed here.

Table 2.3. PURGEABLE STANDARD SOLUTION

Chemical	Density (@ 20°C)	$\frac{\mu g}{mL}$
diethyl ether	0.714	86
cyclopentane	0.746	90
n-dodecane	0.749	90
n-tetradecane	0.763	92
dichloromethane	1.327	160
chloroform	1.483	178
1,2-dichloroethane	1.235	148
2-bromohexane	1.174	141
1-bromo-4-chlorobenzene	1.576	190
1,2,4-trichlorobenzene	1.454	174
p-iodotoluene	1.678	201
benzene	0.878	105
chlorobenzene	1.105	133
tetrachloroethylene	1.623	195
1,3,5-trimethylbenzene	0.894	107
trichloroethylene	1.464	176

of methanol. After adding all compounds invert flask several times to mix. Store at 4°C for no longer than 3 days in the volumetric flask. Aliquots may be transferred to 2 mL ampules and sealed (the transfer should be done at 4°C). Ampules may be stored for up to 4 weeks.

(2) Standard Solution With Internal Standards (Optional). Prepare the standard solution containing internal standards by accurately delivering 3 μL with a microliter syringe of the neat deuterated substances (≥98% chemically and isotopically pure, Table 2.2) into the 25 mL volumetric flask containing purgeable standards (above, 1). Densities are given in Table 2.4. This is done if NBS microcapsules are not available. Store at 4°C for no longer than 3 days in the volumetric flask (see above for transferring to ampules).

Table 2.4. INTERNAL STANDARD SOLUTION (OPTIONAL)

Chemical	Density (@ 20°C)	Level 1		Level 2	
		µL[a]	µg/L[b]	µL[a]	µg/L[b]
d_5-bromoethane	1.460	3	1.4	30	14.0
d_8-naphthalene	1.025	-[c]	1.9	-[d]	9.6
d_5-chlorobenzene	1.106	3	1.1	30	11
2,4,6-d_3-anisole	0.996	3	0.96	30	9.6

[a] µL of neat material added to 25 mL methanol.

[b] µg/L is final concentration in water sample if 2.0 µL of this solution is added to 250 mL.

[c] 6.0 mg is weighed into 25 mL volumetric flask before adding methanol.

[d] 30 mg is weighed into 25 mL volumetric flask before adding methanol.

2.3.3 Internal Standard Solution (If NBS prepared standards are not available)

Prepare deuterated internal standard solution (Table 2.4) by accurately pipetting 3 µL of the deuterated compounds into a 25 mL volumetric flask containing 25 mL of methanol. After all internal standards have been added, invert the flask several times to mix. Store at 4°C for no longer than 3 days in the volumetric flask (see above 2.3.2(1) for long term storage).

2.4 CLEAN-UP PROCEDURES

(1) All glassware to be used, including sample bottles, should be washed with Amway SA-8 laundry detergent, rinsed several times with deionized water, and baked for a minimum of 4 h at 500 to 550°C. All cleaned glassware is immediately capped or covered with precleaned foil to prevent contamination.

(2) The exteriors of the NBS microcapsules containing deuterated internal standards are rinsed with toluene and pentane, air dried at room temperature and stored in a clean screw-capped vial.

(3) Teflon lined septa are sonicated for 10 min in pesticide grade methanol followed by 10 min in pesticide grade pentane and dried in a vacuum oven at 60°C for 1 hr and stored in a screw-capped vial.

2.5 PREPARATION OF CONTROL AND BLANK SAMPLES

(1) Place a clean magnetic stirbar into each of eight 250 mL sample bottles. Place one NBS microcapsule containing the internal standards into each 250 mL sample bottle. If NBS standards are not available, see section 2.6.0(8) for optional internal standard procedures. For drinking water use set No. 1 (Table 2.2). For dirtier waters, set No. 3 may be employed. Fill each bottle with reagent water, leaving no headspace, and seal with Teflon lined septum caps.

(2) Inject through the septum cap of 4 of the bottles with a microliter syringe 2 µL of purgeable standard solution (Table 2.3) to produce the control samples. Inject as far below the septum as possible.

(3) Label and number bottles 1 to 8, 4 as blanks and 4 as controls.

(4) Invert all eight bottles and store at 4°C.

(5) Field controls (2) and field blanks (2) are shipped to the collection site and are handled, stored and shipped in the same manner as field samples (at 4°C). The remaining laboratory blanks and controls are stored at 4°C in the laboratory.

2.6 FIELD COLLECTION

(1) Carry to the field:

(a) Chlorine measurement kit (Hach); 25 mL mixing chamber; Mini Spec 20.

(b) $Na_2S_2O_3$ quenching solution.

(c) Tweezers, pipettes.

(d) Twenty-four NBS microcapsules containing internal standards (Sets 1 or 3, Table 2.2) [see 2.6.0(8): optional].

(e) 2 field control and 2 field blank samples (kept at 4°C).

(f) Twenty-four clean, empty 250 mL sample bottles (which have been capped with septum seals to keep clean) and 24 Teflon lined septa, stored in a clean sample vial. Crimping device, if crimp caps are used. Twenty-four magnetic stirbars (Teflon-coated).

(g) One 3 dram septum capped vial.

(2) A preliminary water sample (~25 mL) is collected at the field site to determine the total chlorine content (only for drinking water, final treated municipal effluent, or water suspected of containing chlorine). This measurement is made with a Bausch and Lomb Mini Spec 20 portable spectrophotometer. The water sample is added to a 25 mL mixing cylinder along with the contents of a DPD Total Chlorine Reagent Powder Pillow. The mixture is shaken and the color is allowed to develop 3 to 6 minutes. Percent transmittance, measured at 530 nm, is converted to mg/L total chlorine using Table 2.5.

(3) Rinse each sample bottle three times with the water sample to be collected and drain, then place a clean magnetic stirbar (handle with clean tweezers) and a NBS microcapsule (use tweezers) containing internal standards in each bottle. Use Set No. 1 (Table 2.2) for drinking water and Set No. 3 for dirtier waters.

Table 2.5. TOTAL CHLORINE (mg/L as Cl) VS. % TRANSMITTANCE

%T	% T Units									
Tens	0	1	2	3	4	5	6	7	8	9
10	4.00	3.84	3.68	3.54	3.42	3.30	3.18	3.08	2.98	2.88
20	2.80	2.71	2.63	2.55	2.48	2.41	2.34	2.28	2.21	2.15
30	2.09	2.04	1.98	1.93	1.88	1.82	1.78	1.73	1.68	1.64
40	1.59	1.55	1.51	1.47	1.43	1.39	1.35	1.31	1.28	1.24
50	1.20	1.17	1.14	1.10	1.07	1.04	1.01	0.98	0.95	0.92
60	0.89	0.86	0.83	0.80	0.78	0.75	0.72	0.70	0.67	0.64
70	0.62	0.60	0.57	0.56	0.52	0.50	0.48	0.46	0.43	0.41
80	0.39	0.37	0.34	0.32	0.30	0.28	0.26	0.24	0.22	0.20
90	0.18	0.16	0.14	0.13	0.11	0.09	0.07	0.05	0.04	0.02

Reprinted from: Water and Wastewater Analysis Procedures Manual, Hach Chemical Co., 1975, p. 2-29.

(4) A stoichiometric quantity (plus 10% excess) of $Na_2S_2O_3$ solution (Figure 2.1) is added to each bottle based upon the total chlorine determination for that sampling point.

(5) Sample bottles are then filled with the water sample, leaving no headspace, and sealed with Teflon lined septa. Remember, 4 duplicates (a duplicate of 4 of the samples) are collected; 2 will serve as surrogate water samples. A 3 dram (12 mL) vial is also filled with sample water and sealed (no internal standards are added). This 12 mL sample will be used for scouting measurements at the laboratory.

(6) All sample bottles are inverted and shipped on ice (e.g., "Blue Ice®") directly to the laboratory by an appropriate air carrier (e.g., Federal Express) in well insulated cartons.

(7) As soon as the samples arrive at the laboratory, spike two of the duplicate water samples with 2 μL (drinking water), or 50 μL (for dirtier waters) of the purgeable standard solution by injecting through the septum cap with a microliter syringe. Inject as far below the septum as possible. These are the surrogate samples.

(8) Break NBS internal standard capsules by placing bottles (all field samples and quality control and assurance samples) on a magnetic stirrer (slightly off center) and using violent stirring conditions completely crush the capsule.

Optional. If NBS microcapsules containing deuterated internal standards are not employed, the internal standards are added (immediately after headspace scouting has been completed, see Analysis Protocol, Chapter 6) by injecting 2 μL of the internal standard solution (Table 2.4) in methanol. Use Level 1 solution for drinking water, Level 2 for dirtier waters. Inject as far below the septum as possible. To insure quantitative transfer of the standards from the syringe to the water, a 10 μL GC-type syringe with a Teflon- tipped plunger is used. The injection technique involves first drawing 2 μL of air, followed by 2.0 μL of standard, followed by a second 2 μL of air. In this way the actual volume of standard to be injected into the sample can be observed in the syringe. After injection of the standard into the sample, an aliquot (~5 μL) of the sample is drawn up into the syringe and expelled back into the sample to insure quantitative introduction of the standards. Mix by placing on a magnetic stirbar plate.

(9) All samples are stored at 4°C until analysis (bottles inverted). Analysis should be performed within 10 days of sample collection.

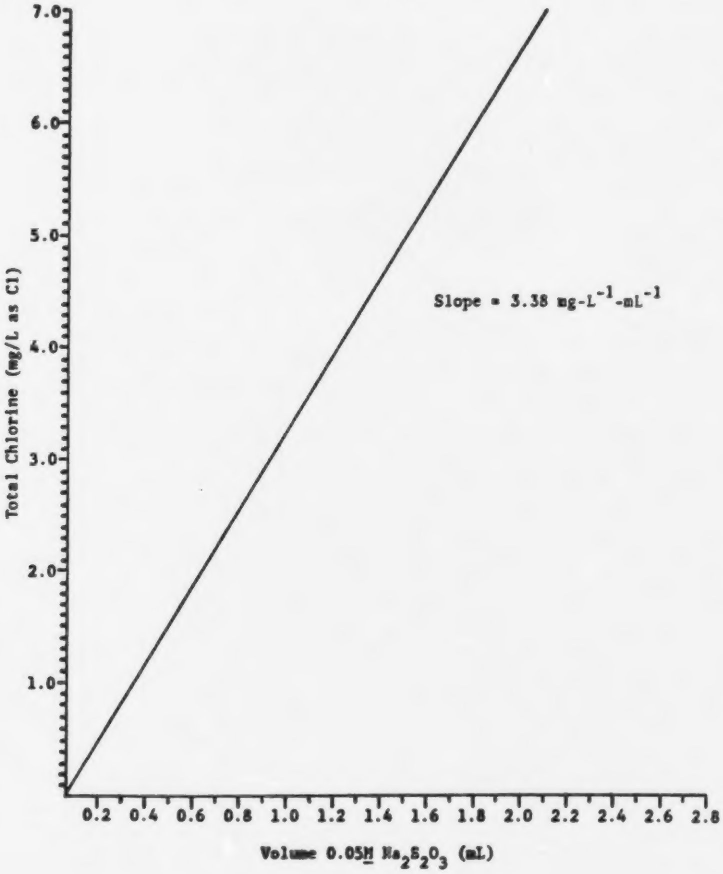

Figure 2.1. Total chlorine vs volume of 0.05M $Na_2S_2O_3$ added to a 1 L sample. (If a 250 mL sample is collected then add 1/4 of the volume indicated).

CHAPTER 3

SAMPLING AND SAMPLE HANDLING FOR NEUTRAL WATER SOLUBLE ORGANICS (NEWS)

3.1 INTRODUCTION

This section describes the collection and handling of drinking water, surface water, and treated municipal and industrial wastewater effluent samples for the analysis of low molecular weight, volatile water soluble compounds. For drinking water, a 200 mL sample will be collected. A 20 mL sample will be collected for all other water types. Immediately after collection, residual chlorine will be quenched using sodium thiosulfate, the sample pH will be adjusted using H_2SO_4 or NaOH, and internal standard solutions will be pipetted into each sample. Samples will be capped, then stored at 4°C until analysis.

Table 3.1 illustrates the sampling regime, including quality control and assurance samples for up to 20 different field samples. Duplicates, surrogates, field and laboratory controls, and field and laboratory blanks are prescribed. The exact number is dependent upon the number of field samples to be collected. For example, if 11 field samples are to be collected, then two duplicates (one duplicate of each of two field samples), one surrogate, and one each of field and laboratory controls and blanks are incorporated as part of the QC/QA Thus, a total of 18 analyses would be performed for 11 field samples (Table 3.1).

Control samples will be prepared by adding known quantities of representative target compounds to reagent (free of NEWS compounds) water. Blanks are unspiked reagent water. Laboratory controls and blanks, after preparation, are stored at 4°C in the laboratory until analysis. Field controls and blanks are shipped to the sampling site and handled in the same manner as field samples in terms of cooling, shipping and storing. Upon the return to the laboratory, representative NEWS target compounds are spiked into replicate field samples (the number of samples treated in this manner is specified in Table 3.1) for use as surrogate samples.

Table 3.1. NUMBER OF QUALITY CONTROL SAMPLES ASSOCIATED WITH FIELD SAMPLES

No. of Samples to be Collected	Duplicates	Surrogates	Controls		Blanks		Total No. of Analyses
			Field	Laboratory	Field	Laboratory	
1	1	-	-	-	1	1	4
2	1	-	-	-	1	1	5
3	1	-	-	-	1	1	6
4	1	-	1	-	1	1	8
5	1	-	1	-	1	1	9
6	1	-	1	-	1	1	10
7	1	-	1	-	1	1	11
8	1	-	1	-	1	1	12
9	1	-	1	-	1	1	13
10	1	-	1	-	1	1	14
11	2	1	1	1	1	1	18
12	2	1	1	1	1	1	19
13	2	1	1	1	1	1	20
14	2	1	1	1	1	1	21
15	2	2	1	1	1	1	23
16	2	2	1	1	1	1	24
17	2	2	1	1	1	1	25
18	2	2	1	1	1	1	26
19	2	2	1	2	2	2	30
20	2	2	2	2	2	2	32

Comparison of the analytical results for controls, blanks and duplicates to results for field samples will provide a measure of uncontrolled contamination and/or analyte losses during sample collection, shipment, and storage, the homogeneity of the sample matrix, and the accuracy and precision associated with sample preparation and analysis. Recovery of internal standards and spiked compounds from the surrogate samples will provide information on matrix effects on method accuracy and precision (if more than two surrogates are included).

If more than 20 samples are to be collected, the additional field samples are matched with the prescribed number of duplicates, surrogates, etc. For example, if 33 samples are to be collected, then lines for 20 and 13 samples, respectively, are consulted and the number of duplicates, surrogates, etc., are a sum of these two values.

3.2 MATERIALS AND REAGENTS

The following materials are required for collecting a set of 20 water samples plus two duplicate and two surrogate samples and two each of field and laboratory controls and blanks for the analysis of neutral water soluble organics (NEWS).

(1) Thirty-two narrow mouth, amber glass, sample bottles with Teflon lined screw caps. For drinking water, 250 mL bottles calibrated to 200 mL are used, for all other water types 25 mL bottles calibrated to 20 mL are used. Bottles are calibrated by adding an accurately measured volume of water, then marking the level of the water miniscus by scratching the outside surface. Bottles should be calibrated before cleaning.

(2) Graduated pipettes (1.0 mL) for preparing and aliquoting standard solutions.

(3) Pasteur pipettes plus pipette bulbs.

(4) One Bausch and Lomb Mini Spec 20 portable spectrometer.

(5) One 25 mL mixing cylinder (Hach Chlorine Determination Kit).

(6) Materials (e.g., "Blue Ice®") for chilling the sample during shipping and storage; insulated shipping containers for samples.

(7) Volumetric flasks (10 mL and 100 mL) for preparing standard solutions.

(8) Syringes for preparing standard solutions - 100 and 250 μL.

(9) Teflon lined screw cap bottles for storing standard solutions.

(10) Narrow range pH paper.

(11) Reagents

  (a) reagent water - reagent water is defined as water in which no interferences are observed at the detection limit for the parameters of interest. A water purification system (Millipore Super-Q® for equivalent) may be used to generate reagent water.

  (b) NBS ampoules containing deuterated NEWS internal standards in water (Table 3.2). If not available prepare as described under Section 3.3.1.

  (c) 10 mL NEWS standards (Table 3.3). Control standards are prepared from pure stock solutions.

  (d) sodium hydroxide - 0.1N in reagent water (must be demonstrated as contaminant free; Chapter 4).

  (e) sulfuric acid solution - 0.1N in reagent water (must be demonstrated as contaminant free; Chapter 4).

  (f) 250 mL $Na_2S_2O_3$ solution - 0.05 M in reagent water (must be demonstrated as contaminant free; Chapter 4).

  (g) DPD Total Chlorine Reagent Powder Pillow (Hach Chemical Co.).

Table 3.2. NBS AMPOULE CONTAINING NEWS INTERNAL STANDARDS IN WATER

	Concentration (μg/Ampoule)[a]	Spiked Concentration (ppb)	
Compound	Set No. 1	200 mL[b] Sample	20 mL[c] Sample
t-Butanol-d_9	112.9	75.3	376
Nitrobenzene-d_5	20.1	13.4	67

[a] Concentration is for the "400" series production run; Set No. 1 contains 7.5 mL water.

[b] 1.0 mL from Set No. 1 is added.

[c] 0.5 mL from Set No. 1 is added.

Table 3.3. NEWS STANDARD SOLUTION

Compound	Density (@ 2°C)	Amount Stock Solution Added (μL to 10 mL water)	Standard Solution (μg/mL in reagent water)
propionitrile	0.782	60	4.6
_n_-butyraldehyde	0.817	15	1.2
dioxane	1.034	30	3.1
cyclopentanone	0.949	20	1.9
hexanol	0.827	40	3.2

3.3 PREPARATION OF STANDARD SOLUTIONS

3.3.1 Internal Standard Solution, Table 3.4 (If NBS standards not available)

(1) With a 50 μL syringe, draw up and accurately weigh 25 mg of _t_-butanol-d_9 and 5 mg of nitrobenzene-d_5 into a 10 mL volumetric flask containing 9 mL of reagent water. Bring to final volume with reagent water and mix well. This is the stock solution.

(2) Transfer this stock solution into a Teflon sealed screw cap bottle. Store at 4°C and protect from light.

(3) With a 250 μL syringe, accurately measure 100 μL of this stock solution and inject into a 20 mL volumetric flask containing 19 mL of reagent water. Make to volume with reagent water and mix well. This is the standard solution.

(4) With a 1 mL graduated pipette, transfer 1.0 mL portions of this solution to 0.5 dram vials. Seal with Teflon lined, septum seal screw caps. Store at 4°C and protect from light.

(5) Fresh standards must be prepared every six months. If signs of degradation or evaporation occur, they should be replaced more frequently.

(6) When compound purity is 96% or greater, the weight can be used without correction to calculate concentration. Compounds which are less than 96% pure cannot be used for standards.

3.3.2 Standard Solutions of NEWS Compounds (Table 3.3)

(1) Prepare a standard stock solution. With a 100 μL syringe, accurately measure the specified volume of each liquid standard

Table 3.4. DEUTERATED INTERNAL STANDARD SOLUTION (OPTIONAL)

Compound	Standard Concentration (μg/mL)	Spiked Concentration (ppb) 200 mL[a] Sample	 20 mL[b] Sample
t-Butanol-d_9	12.5	62	620
Nitrobenzene-d_5	2.5	12	120

[a] Concentration when 1 mL of solution is spiked into a 200 mL water sample.

[b] Concentration when 1 mL of solution is spiked into a 20 mL water sample.

(Table 3.3) into a 10 mL volumetric flask containing 9 mL water. Make to volume with reagent water and mix well. Transfer into a Teflon sealed screw cap bottle. Store 4°C and protect from light.

(2) Prepare the standard solution. With a 250 μL syringe, accurately measure 100 μL of the control stock solution into a 100 mL volumetric flask. Dilute to volume with reagent water and mix well. Transfer into a Teflon sealed screw cap bottle. Store at 4°C and protect from light.

(3) Fresh standards must be prepared every six months. If signs of degradation or evaporation occur, they should be replaced more frequently.

(4) When compound purity is 96% or greater, the weight can be used without correction to calculate concentration. Compounds which are less than 96% pure cannot be used for standards.

3.4 GLASS CLEANING PROCEDURES

(1) All glassware to be used including sample bottles should be washed with Amway SA-8 laundry detergent rinsed several times with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with foil (precleaned with hexane) to prevent contamination.

(2) Teflon liners and Teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane.

3.5 PREPARATION OF CONTROL AND BLANK SAMPLES

(1) Aliquot reagent water into eight sample bottles. For drinking water 200 mL samples will be placed in 250 mL bottles, for all other water types, 20 mL will be aliquoted into 25 mL bottles. Number the bottles 1 to 4.

(2) Measure total chlorine in a separate water sample and, if necessary, quench using sodium thiosulfate (Section 6, step 2 and 3).

(3) Spike all samples with 1 mL (0.5 mL for 20 mL sample) of internal standard solution.

(4) Spike and label the sample bottles as described in Table 3.5.

(5) Adjust the sample pH to 6-7 in all bottles using sulfuric acid or sodium hydroxide solution. Use narrow range pH paper to measure pH.

(6) Field controls and field blanks are shipped to the collection site and are handled, stored and shipped in the same manner as field samples. Laboratory blanks and controls are stored at 4°C in the laboratory.

3.6 FIELD COLLECTION

(1) Water samples (20 field samples plus two duplicate and two surrogates) are collected by rinsing sample bottles three times

Table 3.5. CONTROL AND BLANK SAMPLES

Bottles	Amount Spiked[a]	Disposition	Sample Type
1,2	1 mL	send to field	field control
3,4	1 mL	store in lab	laboratory control
5,6	none	send to field	field blank
7,8	none	store in lab	laboratory blank

[a] Spiked with standard NEWS compounds (Table 3.3).

with sample water then filling to the calibration mark. Drinking water samples are collected (200 mL) in 250 mL bottles. For all other water types 20 mL are collected in 25 mL bottles.

(a) Addition of NBS Standards--The deuterated internal standards are pipetted (use 1.0 mL pipet) into each water sample using volumes given in Table 3.2.

(b) Addition of Deuterated Internal Standards (Optional if NBS ampoules not available)--Internal standard solutions (1.0 mL) in 0.5 dram vials (Table 3.4) are added to each water sample. Rinse the vials three times with sample water and add to sample bottle.

(2) A preliminary water sample (~25 mL) is collected for the purpose of determining total chlorine content. This measurement is made at the sample collection site and utilizes the Bausch and Lomb Mini Spec 20 portable spectrophotometer. The water sample is added to a 25 mL mixing cylinder along with the contents of a DPD Total Chlorine Reagent Powder Pillow (Hach Chemical Co.). The mixture is shaken and the color is allowed to develop for 3 to 6 minutes. Percent transmittance, measured at 530 nm, is converted to mg/L total chlorine using Table 3.6.

(3) Residual chlorine is quenched by adding a stoichiometric quantity of $Na_2S_2O_3$ (Figure 3.1) to the bottles containing the water sample by delivering the appropriate volume of the 0.05M solution.

(4) Adjust sample pH to 6 to 7 with sodium hydroxide or sulfuric acid. Use narrow range pH paper to measure pH.

(5) Samples should be shipped on ice (e.g., "Blue Ice®") directly to the laboratory by an appropriate air carrier (e.g., Federal Express) in well insulated cartons.

(6) As soon as the samples arrive at the laboratory spike the two surrogate samples with 1.0 mL of the NEWS standard solution (Table 3.3) using a 1 mL graduated pipette.

(7) All samples should be stored at 4°C until analysis. All samples should be processed within 21 days of collection.

Table 3.6. TOTAL CHLORINE (mg/L as Cl) VS. % TRANSMITTANCE

%T	% T Units									
Tens	0	1	2	3	4	5	6	7	8	9
10	4.00	3.84	3.68	3.54	3.42	3.30	3.18	3.08	2.98	2.88
20	2.80	2.71	2.63	2.55	2.48	2.41	2.34	2.28	2.21	2.15
30	2.09	2.04	1.98	1.93	1.88	1.82	1.78	1.73	1.68	1.64
40	1.59	1.55	1.51	1.47	1.43	1.39	1.35	1.31	1.28	1.24
50	1.20	1.17	1.14	1.10	1.07	1.04	1.01	0.98	0.95	0.92
60	0.89	0.86	0.83	0.80	0.78	0.75	0.72	0.70	0.67	0.64
70	0.62	0.60	0.57	0.56	0.52	0.50	0.48	0.46	0.43	0.41
80	0.39	0.37	0.34	0.32	0.30	0.28	0.26	0.24	0.22	0.20
90	0.18	0.16	0.14	0.13	0.11	0.09	0.07	0.05	0.04	0.02

Reprinted from: Water and Wastewater Analysis Procedures Manual, Hach Chemical Co., 1975, p. 2-29.

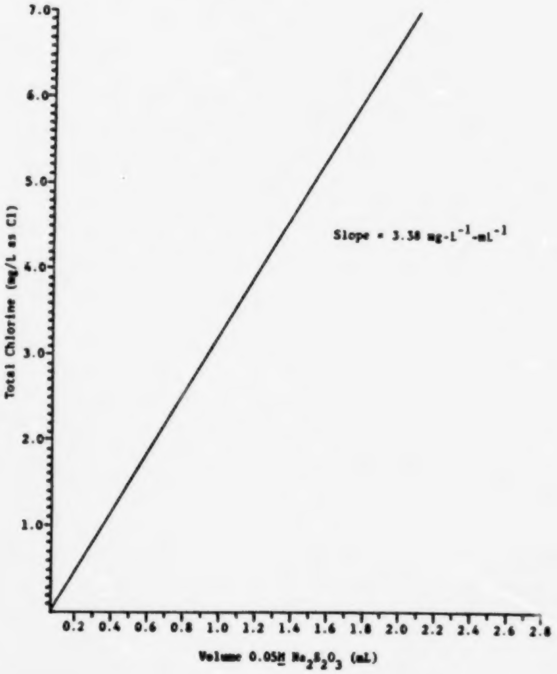

Figure 3.1. Total chlorine vs volume of 0.05M $Na_2S_2O_3$ added to a 1 L sample. (If a 200 mL sample is collected then add 1/5 of the volume indicated).

CHAPTER 4

SAMPLING AND SAMPLE HANDLING FOR EXTRACTABLE ORGANICS (ESSA, WABN)

4.1 INTRODUCTION

This section describes the collection and handling of drinking water, surface water, and treated municipal and industrial wastewater effluent samples for the analyses of solvent-extractable compounds. Separate samples will be collected for the analysis of acid extractable compounds (ESSA) and pH 8 extractable compounds (WABN). For drinking water, a 11.35 L sample will be collected for pH 8 extractable compounds only. For all other analyses a 1 L sample will be collected. Immediately after collection, internal standard solutions will be added to each sample. At the same time, residual chlorine will be quenched using sodium thiosulfate, the sample pH will be adjusted using H_2SO_4 or NaOH, and the volumes of each recorded. Methylene chloride (2%, v/v) will be added as a preservative for all ESSA samples and WABN drinking water samples. A "keeper" solvent (60 mL of 35% methylene chloride, 65% hexane) will be added to the other WABN samples. Samples will be capped, then stored at 4°C until analysis.

Table 4.1 illustrates the sampling regime with quality control samples for 20 different field samples. Duplicates, surrogates, field and laboratory controls, field and laboratory blanks, and procedural blanks are prescribed. This regime is for WABN or ESSA samples; quality control samples are repeated for each sample type. The exact number is dependent upon the number of field samples to be collected. For example, if 11 field samples are to be collected, then two duplicates (one duplicate of each of two field samples), one surrogate, one each of field and laboratory controls and blanks, and three procedural blanks are incorporated as part of the QC/QA. Thus, a total of 21 analyses would be performed for 11 field samples (Table 4.1). If both WABN and ESSA fractions are analyzed, a

Table 4.1 NUMBER OF QUALITY CONTROL SAMPLES ASSOCIATED WITH FIELD SAMPLES

No. of Samples to be Collected	Duplicates	Surrogates	Controls		Blanks			Total No. Analyses
			Field	Laboratory	Field	Laboratory	Procedural	
1	1	-	-	-	1	1	1	5
2	1	-	-	-	1	1	1	6
3	1	-	1	-	1	1	1	8
4	1	-	1	-	1	1	1	9
5	1	-	1	-	1	1	2	11
6	1	-	1	-	1	1	2	12
7	1	-	1	-	1	1	2	13
8	1	-	1	-	1	1	2	14
9	1	-	1	-	1	1	2	15
10	1	1	1	1	1	1	2	18
11	2	1	1	1	1	1	3	21
12	2	1	1	1	1	1	3	22
13	2	1	1	1	1	1	3	23
14	2	1	1	1	1	1	3	24
15	2	2	1	1	1	1	3	26
16	2	2	1	1	1	1	3	27
17	2	2	1	1	1	1	4	29
18	2	2	1	1	1	1	4	30
19	2	2	1	2	2	2	4	34
20	2	2	2	2	2	2	4	36

total of 42 analyses would be required. An exception to this schedule is prescribed for wastewater samples where two 1 L field samples are collected for each WABN sample in addition to the QC samples in Table 4.1. Only one of these is analyzed unless the sample is found to be emulsion prone (Chapter 9 Part II, section 9.4).

Control samples will be prepared by adding known quantities of representative target compounds to reagent water (free of ESSA and WABN compounds). Blanks are unspiked reagent water. Laboratory controls and blanks, after preparation, are stored at 4°C in the laboratory until analysis. Field controls and blanks are shipped to the sampling site and handled in the same manner as field samples in terms of cooling, shipping, and storing. Upon return to the laboratory, representative ESSA or WABN target compounds are spiked into replicate field samples (the number of samples treated in this manner is specified in Table 4.1) for use as surrogate samples.

Comparison of the analytical results for controls, blanks and duplicates will provide a measure of uncontrolled contamination and/or analyte losses during sample collection, shipment, and storage, the homogeneity of the sample matrix, and the accuracy and precision associated with sample preparation and analysis. Recovery of internal standards and spiked compounds from the surrogate samples will provide information on matrix effects on method accuracy and precision (if two or more surrogates are included).

If more than 20 samples are to be collected, the additional field samples are matched with the prescribed number of duplicates, surrogates, etc. For example, if 33 samples are to be collected, then lines for 20 and 13 samples, respectively, are consulted and the number of duplicates, surrogates, etc., are a sum of these two.

4.2 MATERIALS AND REAGENTS

The following materials are required for collecting a set of 20 water samples plus two duplicate samples and two surrogate samples and two each of field and laboratory controls, and two each of field and laboratory blanks for the analysis of both ESSA and WABN compounds.

(1) Fifty-six (eighty-four, if all samples are wastewaters) narrow mouth glass sample bottles with Teflon lined screw-caps. For drinking water, three one gallon bottles are used to collect each WABN sample, for all other water types, one quart bottles are used (up to eighty-four one gallon bottles and twenty-eight one quart bottles may be required). Each sample bottle is calibrated by filling with 3.78 L of reagent water for a 1 gal container and 940 mL of reagent water for a 1 quart container. The water level is marked on the bottle and then the sampling containers are emptied.

(2) Twenty 20 mL polyethylene or polypropylene vials for scouting samples.

(3) 10 mL graduated pipettes.

(4) Pasteur pipettes plus pipette bulbs.

(5) One Bausch and Lomb Mini Spec 20 portable spectrometer.

(6) One 25 mL mixing cylinder (Hach Chlorine Determination Kit).

(7) Materials for chilling the sample during shipping and storage - insulated shipping containers and "Blue Ice®".

(8) Volumetric flasks (1000, 500, 100, and 10 mL) for preparing standard solution

(9) Teflon-lined screw-cap bottles for storing standard solutions

(10) Graduated cylinders - 1000 mL, 50 mL.

(11) Syringes for preparing standard solutions - 100, 250, and 1000 µL.

(12) One hundred 3 dram vials with 15 mm screw caps (Supelco 3-3112) and Teflon lined rubber septa (Supelco 3-3115).

(13) Teflon lined rubber septa (Supelco 3-3115).

(14) Reagents

  (a) reagent water - reagent water is defined as water in which no interferences are observed at the detection limit for the parameters of interest. A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

  (b) 5.0 mL of each internal standard solution (for use if NBS ampoules not available; Tables 4.2 and 4.3). Set 1

Table 4.2. INTERNAL STANDARD SOLUTSIONS - ESSA

Compound	Concentration (μg/5 mL in methanol)[a] Set 1	Concentration (μg/5 mL in methanol)[a] Set 2	Spiked Concentration (ppb) Set 1	Spiked Concentration (ppb) Set 2
benzoic acid-d_5	1	100	1	100
heptanoic acid-d_{13}	15	100	15	100

[a] Also, μg spiked into 1 L of sample.

standards are used for spiking drinking water and other relatively clean samples. Set 2 standards are used for spiking municipal and industrial wastewaters. Internal standard solutions can be prepared from pure certified standards or certified solutions of standards.

(c) 10 mL each standard solution (Table 4.4 and 4.5) for ESSA and WABN. Standards are prepared from pure stock solutions.

(d) sodium hydroxide solution (10N) - dissolve 40 g NaOH in reagent water and dilute to 100 mL.

(e) sulfuric acid solution (1+1) - slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

(f) 250 mL $Na_2S_2O_3$ solution - 0.05 M in reagent water.

(g) DPD Total Chlorine Reagent Powder Pillow (Hach Chemical Co.).

(h) methylene chloride - distilled in glass (Burdick and Jackson).

(i) acetone - distilled in glass (Burdick and Jackson).

(j) methanol - distilled in glass (Burdick and Jackson).

(k) 35% methylene chloride in hexane (v/v) - distilled in glass (Burdick and Jackson).

(l) NBS ampoules containing deuterated ESSA and WABN internal standards in methanol (Tables 4.6 and 4.7); optional.

4.3 PREPARATION OF STANDARD SOLUTIONS

4.3.1 Set 1 ESSA Internal Standard Solutions - Table 4.2 (For use if NBS ampoules are not available; for drinking and surface waters)

Table 4.3. INTERNAL STANDARD SOLUTIONS - WABN

		Set No. 1				Set No. 2		
				Spiked Concentration (ppb)				
Compound	Density (@ 20°C)	Volume (μL) or Weight (mg) Added to 500 mL	μg/mL in Methanol	Drinking Water[a]	Surface Water[b]	Volume (μL) or Weight (mg) Added to 500 mL	μg/5 mL[c] in Methanol	Spiked Concentration (ppb)
o-Xylene-d_{10}	0.98	3	5.9	1.6	2.6	10	98	104
Nitrobenzene-d_5	1.25	100	250	66	110	40	500	530
Naphthalene-d_8	1.06	20	42	11	19	50	530	560
Acridine-d_9	S	20	40	11	18	10	100	106
Phenol-d_6	S	100	200	53	88	40	400	420
Propiophenone-d_5	1.05	-	-	-	-	10	105	110
1-Phenyl-d_5-ethanol	1.06	-	-	-	-	50	530	560
Perylene-d_{12}	S	1	2	0.53	0.88	10	100	110
Acetophenone-d_5	1.07	-	-	-	-	10	107	110

[a] 1.0 mL is added to each 1 gal aliquot of sample (3 gal/sample total).

[b] 1.0 mL is added to 940 mL of sample.

[c] Amount added to 940 mL of municipal, industrial or energy effluent sample.

Table 4.4. STANDARD SOLUTION FOR ESSA

Compound	Densities (@ 20°C)	Concentration[a] μg/mL
2,3,6-trichlorophenol	S[b]	500
pentachlorophenol	S	500
n-nonanoic acid	0.906	450
n-decanoic acid	S	500
dichloroacetic acid	1.563	780
trichloroacetic acid	S	500
2,4,5-trichlorophenoxyacetic acid	S	500
benzoic acid	S	500
p-toluic acid	S	500
p-nitrobenzoic acid	S	500
3,4-dichlorobenzoic acid	S	500
o-methoxybenzoic acid	S	500
2,5-dinitrophenol	S	500
o-mercaptobenzoic acid	S	500

[a] Concentration in the secondary dilution.

[b] Solid.

(1) Accurately weigh 10.0 mg of benzoic acid - d_5 and 150 mg of heptanoic acid - d_{13} into a 500 mL volumetric flask. Dissolve these materials in methanol and dilute to the mark.

(2) Transfer this stock solution into a Teflon sealed screw cap bottle. Store at 4°C and protect from light.

(3) Accurately measure 5.0 ml of this stock solution and transfer to a 500 mL volumetric flask. Dilute to volume with methanol and mix well.

(4) Transfer 5.0 mL portion of this solution to 3 dram vials. Seal with Teflon lined, septum seal, screw caps. Store at 4°C and protect from light.

Table 4.5. STANDARD SOLUTION FOR WABN

Compound	Densities (@ 20°C)	Concentration μg/mL[a]
pyridine	0.978	490
α-picoline	0.943	470
2-(*n*-butoxy)ethanol	0.903	450
benzaldehyde	1.044	520
aniline	1.022	510
2,3,5-trimethylpyridine	0.917	460
acetophenone	1.030	520
2,3-dihydrobenzofuran	1.065	530
o-cresol	S[b]	500
isophorone	0.923	460
1,2,4-trichlorobenzene	1.454	720
m-chloroaniline	1.206	600
α-terpineol	0.933	470
tributylamine	0.778	390
2-nitrocresol	S	500
n-decanol	0.829	410
4-chlorocresol	S	500
p-*t*-butylphenol	S	500
n-tridecane	0.756	380
nicotine	1.010	500
2,4-dimethylquinoline	S	500
dimethylphthalate	1.190	600
n-pentadecane	0.769	380
1,2-dichloronaphthalene	S	500
diphenylamine	S	500
tributylphosphate	0.979	490
n-heptadecane	0.777	390
anthracene	S	500
n-octadecane	0.777	390
dibutylphthalate	1.043	520
n-eicosane	S	500
pyrene	S	500
perylene	S	500

[a] Concentration in secondary dilution.

[b] Solid.

Table 4.6. NBS AMPOULES CONTAINING ESSA INTERNAL STANDARDS IN METHANOL

	Concentration (μg/Ampoule)[a]		Spiked Concentration (ppb)	
Compound	Set No. 1	Set No. 2	Set No. 1[b]	Set No. 2[c]
Benzoic-d_5 acid	2.15	200.6	2.15	200.6
Heptanoic-d_{13} acid	25.1	202.9	25.1	202.9

[a] Concentration in the "400" series production run; each ampoule contains 5.0 mL methanol.

[b] For drinking and surface waters.

[c] For municipal, industrial and energy effluents.

Table 4.7. NBS AMPOULES CONTAINING WABN INTERNAL STANDARDS IN METHANOL

	Concentration (μg/ampoule)[a]		Spiked Concentration (ppb)		
			Set No. 1		
Compound	Set No. 1	Set No. 2	Drinking Water[b]	Surface Water[c]	Set No. 2[d]
o-Xylene-d_{10}	2.8	104.7	0.74	3.0	111
Nitrobenzene-d_5	94.2	538.8	24.9	100	573
Naphthalene-d_8	20.2	500.7	5.3	21.5	533
Acridine-d_9	19.8	100.3	5.2	21.1	107
Phenol-d_6	112.7	400.4	29.8	119.9	426
Propiophenone-d_5	---[e]	98.1	---	---	104
1-Phenyl-d_5-ethanol	---	520.9	---	---	554
Perylene-d_{12}	1.1	98.1	0.29	1.2	104
Acetophenone-d_5	---	104.7	---	---	111

[a] Concentration in the "400" series production run; each ampoule contains 5.0 mL methanol.

[b] For drinking water, 1 ampoule is added to each 1 gal of sample (3 gal or 11.35 L total per sample).

[c] For surface water, 1 ampoule is added per 940 mL of sample.

[d] For municipal, industrial, and energy effluent samples, 1 ampoule is added per 940 mL of sample.

[e] Not present.

(5) Fresh standards must be prepared every six months. If signs of degradation or evaporation occur they should be replaced more frequently.

(6) When compound purity is 96% or greater, the weight (or volume) can be used without correction to calculate concentration. Compounds that are less than 96% pure cannot be used for standards.

4.3.2 Set 2 ESSA Internal Standard Solutions - Table 4.2 (For use if NBS ampoules are not available; for industrial and energy wastewater effluents)

(1) Accurately weigh 100 mg of benzoic acid - d_5 and 100 mg of heptanoic acid - d_{13}; dissolve in methanol in a 500 mL volumetric flask. Dilute the sample to volume with methanol and mix well.

(2) Accurately measure 50 mL of this stock solution and transfer to a 500 mL volumetric flask. Dilute to the mark with methanol and mix well.

(3) Transfer 5.0 mL portions of the dilute solution into 3 dram vials. Seal with Teflon lined, septum seal screw caps. Store at 4°C and protect from light.

(4) Prepare fresh standards every six months. If signs of degradation or evaporation occur they should be replaced more frequently.

4.3.3 Set 1 WABN Internal Standard Solutions - Table 4.3 (For use if NBS ampoules are not available; for drinking and surface waters)

(1) Accurately weigh the amount of solid compounds indicated in Table 4.3 for Set 1 and dissolve in methanol in a 500 mL volumetric flask. With a 100 μL syringe accurately measure the volumes of the liquid standards into the same flask. Dilute the stock solution to the mark with methanol and mix well.

(2) Transfer this stock solution into a Teflon sealed screw cap bottle. Store at 4°C and protect from light.

(3) Accurately measure 50 mL of this stock and transfer to a 500 mL volumetric flask. Dilute to volume with methanol and mix well.

(4) Transfer 5.0 mL portion of this solution to 3 dram vials. Seal with Teflon lined, septum seal, screw caps. Store at 4°C and protect from light.

(5) Fresh standards must be prepared every six months. If signs of degradation or evaporation occur they should be replaced more frequently.

(6) When compound purity is 96% or greater, the weight can be used without correction to calculate concentration. Compounds which are less than 96% pure cannot be used for standards.

4.3.4 Set 2 WABN Internal Standard Solutions - Table 4.3 (For use if NBS ampoules are not available; for industrial and energy wastewater effluents)

(1) Accurately weigh the amount of solid compounds indicated in Table 4.3 for Set 2 and dissolve in methanol in a 500 mL volumetric flask. With a 500 µL syringe accurately measure the volumes of the liquid standards into the same flasks. Dilute the stock solution to the mark with methanol and mix well.

(2) Accurately measure 50 mL of this stock solution and transfer to a 500 mL volumetric flask. Dilute to the mark with methanol and mix well.

(3) Transfer 5.0 mL portions solution into 3 dram vials. Seal with Teflon lined, septum seal screw caps. Store at 4°C and protect from light.

(4) Prepare fresh standards every six months. If signs of degradation or evaporation occur they should be replaced more frequently.

4.3.5 Standard Solutions (Tables 4.4 and 4.5)

(1) Prepare individual stock solutions (50 mg/mL) for each standard compound.

- Solids - accurately weigh about 0.500 grams of pure material. Dissolve the material in acetone, dilute to volume in a 10 mL volumetric flask.
- Liquids - with a 1000 µL syringe accurately add 500 µL of pure material to methanol (Tables 4.4 and 4.5) in a 10 mL volumetric flask. Dilute to volume.

When compound purity is 96% or greater, the weight can be used without correction to calculate the concentration of stock standards. Compounds which are less than 96% pure cannot be used for standards.

(2) Transfer stock solutions into a Teflon sealed screw cap bottle. Store at 4°C and protect from light. Prepare fresh solutions every six months. If signs of degradation or evaporation occur, they should be replaced more frequently.

(3) Prepare standard solutions individually for ESSA and WABN compounds as shown in Tables 4.4 and 4.5. With a 250 μL syringe, accurately add 100 μL of each stock standard to a 10 mL volumetric flask and dilute to volume with methanol. Transfer to a Teflon sealed screw cap bottle. Store at 4°C and protect from light.

4.4 GLASS CLEANING PROCEDURES

(1) All glassware to be used, including sample bottles should be washed with Amway S-A-8 laundry detergent, rinsed several times with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with precleaned foil to prevent contamination.

(2) Teflon liners and Teflon lined septa are sonicated for 10 minutes in methanol followed by 10 minutes in pesticide grade pentane. Store in a clean vial.

4.5 PREPARATION OF CONTROL AND BLANK SAMPLES

(1) Dispense aliquots of reagent water into sixteen sample bottles. For drinking water, 3.50 L samples will be placed in 1 gallon bottles. Number the bottles 1 to 16.

(2) Measure total chlorine in the water sample and, if necessary, quench using sodium thiosulfate (Section 4.6, step 3).

(3) Spike samples 1-8 with WABN internal standard solutions (Table 4.3) and 9-16 with ESSA internal standards (Table 4.2). If drinking or surface water samples are to be collected spike with 5.0 mL of set 1 standard (1 vial of set 1 internal standard). If municipal, industrial or energy effluents are to be collected spiked with 5.0 mL of set 2 standards (1 vial of set 2). If NBS standards are available, see 4.6(1)(a) below.

(4) Spike with standard solutions (Tables 4.4 and 4.5) and label sample bottles as described in Table 4.8.

Table 4.8. CONTROL AND BLANK SAMPLES

Bottle	Spike	Handle	Sample pH	Sample Type
1 & 2	1.0 mL WABN[a] standard solution	send to field	6-8	field control WABN
3 & 4	1.0 mL WABN standard solution	store in lab	6-8	laboratory control WABN
5 & 6	none	send to field	6-8	field blank WABN
7 & 8	none	store in lab	6-8	laboratory blank WABN
9 & 10	1.0 mL ESSA[b] standard solution	send to field	4-5	field control ESSA
11 & 12	1.0 mL ESSA standard solution	store in lab	4-5	laboratory control ESSA
13 & 14	none	send to field	4-5	field blank ESSA
15 & 16	none	store in lab	4-5	laboratory blank ESSA

[a] pH 8 extractable organics

[b] acid extractable organics

(5) Adjust the sample pH in all bottles as specified in Table 4.8 using sulfuric acid or sodium hydroxide solution. Use narrow range pH paper to measure pH.

(6) Add 60 mL of 35% (v/v) methylene chloride in hexane to all WABN samples except drinking water, where 2% (v/v) methylene chloride is added. Add 2% (v/v) methylene chloride to all ESSA samples. Cap bottles and mix well.

(7) Field controls and field blanks are shipped to the collection site and are handled, stored, and shipped in the same manner as field samples. Laboratory blanks and controls are stored at 4°C in the laboratory.

4.6 FIELD COLLECTION

(1) Water samples are collected by rinsing sample bottles three times with sample water, then filling to the calibration mark.

  (a) Addition of NBS Standards--For drinking and surface waters, 5.0 mL (1 ampoule) of Set No. 1 standard is added to the appropriate volume of water (see Tables 4.6 and 4.7). Municipal, industrial, and energy wastewater effluent samples are spiked with 5.0 mL of Set No. 2 (Table 4.7). In either case rinse the vials containing the internal standard solutions three times with sample water and add to the sample bottle.

  (b) Addition of Deuterated Internal Standards (Optional if NBS ampoules are not available)--Proceed as in (a) above, using internal standard solutions prepared in Sections 4.3.1, 4.3.2, 4.3.3, or 4.3.4 (see Tables 4.2 and 4.3 for amounts).

(2) A preliminary water sample (~25 mL) is collected for the purpose of determining total chlorine content. This measurement is made at the sample collection site and utilizes the Bausch and Lomb Mini Spec 20 portable spectrophotometer. The water sample is added to a 25 mL mixing cylinder along with the contents of a DPD Total Chlorine Reagent Powder Pillow (Hach Chemical Co.). The mixture is shaken and the color is allowed to develop for 3 to 6 minutes. Percent transmittance, measured at 530 nm, is converted to mg/L total chlorine using Table 4.9.

Table 4.9. TOTAL CHLORINE (mg/L as Cl) VS. % TRANSMITTANCE

%T	% T Units									
Tens	0	1	2	3	4	5	6	7	8	9
10	4.00	3.84	3.68	3.54	3.42	3.30	3.18	3.08	2.98	2.88
20	2.80	2.71	2.63	2.55	2.48	2.41	2.34	2.28	2.21	2.15
30	2.09	2.04	1.98	1.93	1.88	1.82	1.78	1.73	1.68	1.64
40	1.59	1.55	1.51	1.47	1.43	1.39	1.35	1.31	1.28	1.24
50	1.20	1.17	1.14	1.10	1.07	1.04	1.01	0.98	0.95	0.92
60	0.89	0.86	0.83	0.80	0.78	0.75	0.72	0.70	0.67	0.64
70	0.62	0.60	0.57	0.56	0.52	0.50	0.48	0.46	0.43	0.41
80	0.39	0.37	0.34	0.32	0.30	0.28	0.26	0.24	0.22	0.20
90	0.18	0.16	0.14	0.13	0.11	0.09	0.07	0.05	0.04	0.02

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(3) Residual chlorine is quenched by adding a stoichiometric quantity of 0.05 M $Na_2S_2O_3$ (Figure 4.1, a 10% excess is used and is already incorporated here) to the bottle containing the aqueous sample.

(4) Samples collected for the analysis of WABN compounds are adjusted to pH 6 to 8. Samples collected for the analysis of ESSA compounds are adjusted pH 4 to 5. Use narrow range pH paper to measure pH. Use sodium hydroxide or sulfuric acid for pH adjustment. (Note: verify contamination free as described in Chapter 9.)

(5) Add 60 mL of 35% (v/v) methylene chloride in hexane to all WABN samples except drinking water as a preservative, cap the samples, and shake well. Add 2% (v/v) methylene chloride to WABN drinking water samples and ESSA samples. The samples must be maintained at 4°C from the time of collection until extraction.

(6) Samples should be shipped on ice (e.g., "Blue Ice®") directly to the laboratory by an appropriate air carrier (e.g., Federal Express) in well insulated cartons.

(7) As soon as the samples arrive at the laboratory, the surrogate samples must be spiked with target compounds. Samples for the analyses of extractable strong acids (ESSA) and weak acids, bases and neutrals (WABN) are spiked with the corresponding standard spiking solutions (Tables 4.4 and 4.5). Drinking water and surface water samples are spiked with 100 µL of the appropriate ESSA standard solution into a 940 mL of sample using a 250 µL syringe. Industrial, municipal and energy wastewaters are spiked with 1 mL of the appropriate ESSA standard solution into 940 mL of sample using a 1 mL graduated pipette.

For WABN compounds, 100 µL of the WABN standard solution is spiked into each 1 gal of drinking water and 940 mL of surface water. The municipal, industrial, and industrial effluent samples are spiked with 1.0 mL of the WABN standard solution (940 mL of sample).

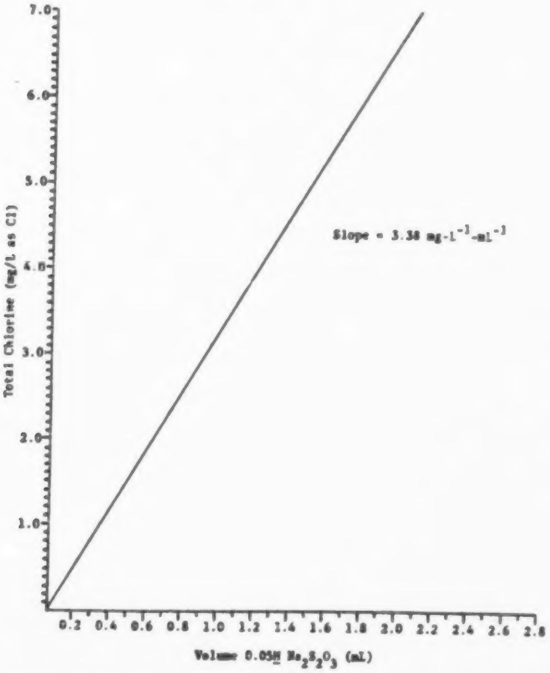

Figure 4.1. Total chlorine vs volume of 0.05M $Na_2S_2O_3$ added to a 1 L sample. (If a 11.35 L sample is collected, 11.35x the amount of $Na_2S_2O_3$ must be added.)

CHAPTER 5

SAMPLING AND SAMPLE HANDLING FOR OTHER IONIC COMPOUNDS (VOSA, NOVA, SAM)

5.1 INTRODUCTION

This section describes the collection and handling of drinking water, surface waters, and treated municipal and industrial wastewater effluent samples for the analysis of ionic "intractable" compounds. Separate samples will be collected for the analysis of low molecular weight carboxylic acids (VOSA), nonvolatile organic acids (NOVA), and amines (SAM). For drinking water, and surface water, a 3.5 L sample will be collected. For all other water types, a 1 L sample will be collected. Immediately after collection, internal standard solutions will be pipetted into each sample. At the same time, residual chlorine will be quenched using sodium thiosulfate, the sample pH will be adjusted using H_2SO_4 or NaOH, and 2% (v/v) methylene chloride will be added as a preservative. Samples will be capped, then stored at 4°C until analysis.

Table 5.1 illustrates the sampling regime with quality control samples for up to 20 different field samples. Duplicates, surrogates, field and laboratory controls, and field, laboratory, and procedural blanks are prescribed. This regime is for VOSA, NOVA, or SAM samples; quality control samples are repeated for each sample type. The exact number of controls is dependent upon the number of field samples to be collected. For example, if 11 field samples are to be collected, then two duplicates (one duplicate of each of two field samples), one surrogate, and one each of field and laboratory controls and blanks are incorporated as part of QC. During sample processing three procedural blanks would also be run. Thus, a total of 21 analyses would be performed for 11 field samples (Table 5.1). If VOSA, NOVA, and SAM fractions are all analyzed, a total of 63 analyses would be required.

Table 5.1. NUMBER OF QUALITY CONTROL SAMPLES ASSOCIATED WITH FIELD SAMPLES

No. of Samples to be Collected	Duplicates	Surrogates	Controls		Blanks			Total No. Analyses
			Field	Laboratory	Field	Laboratory	Procedural	
1	1	-	-	-	1	1	1	5
2	1	-	-	-	1	1	1	6
3	1	-	1	-	1	1	1	7
4	1	-	1	-	1	1	1	9
5	1	-	1	-	1	1	2	11
6	1	-	1	-	1	1	2	12
7	1	-	1	-	1	1	2	13
8	1	-	1	-	1	1	2	14
9	1	-	1	-	1	1	2	15
10	1	1	1	1	1	1	2	18
11	2	1	1	1	1	1	3	21
12	2	1	1	1	1	1	3	22
13	2	1	1	1	1	1	3	23
14	2	1	1	1	1	1	3	24
15	2	2	1	1	1	1	3	25
16	2	2	1	1	1	1	3	27
17	2	2	1	1	1	1	4	29
18	2	2	1	1	1	1	4	30
19	2	2	1	2	2	2	4	34
20	2	2	2	2	2	2	4	36

Control samples will be prepared by adding known quantities of representative target compounds to reagent (free of VOSA, NOVA, and SAM compounds) water. Blanks are unspiked reagent water. Laboratory controls and blanks, after preparation, are stored at 4°C in the laboratory until analysis. Field controls and blanks are shipped to the sampling site and handled in the same manner as field samples in terms of cooling, shipping and storing. Upon return to the laboratory, representative VOSA, NOVA, or SAM target compounds are spiked into replicate field samples (the number of samples treated in this manner is specified in Table 5.1) for use as surrogate samples.

Comparison of the analytical results for controls, blanks and duplicates will provide a measure of uncontrolled contamination and/or analyte losses during sample collection, shipment, and storage, the homogeneity of the sample matrix, and the accuracy and precision associated with sample preparation and analysis. Recovery of internal standards and spiked compounds from the surrogate samples will provide information on matrix effects on method accuracy and precision (if two or more surrogates are included).

If more than 20 samples are to be collected, the additional field samples are matched with the prescribed number of duplicates, surrogates, etc. For example, if 33 samples are to be collected, then lines for 20 and 13 samples, respectively, are consulted and the number of duplicates, surrogates, etc., are a sum of these two.

5.2 MATERIALS AND REAGENTS

The following materials are required for collecting a set of 20 water samples plus two duplicate and two surrogate samples and two each of field and laboratory controls, and two each of field and laboratory blanks for the analysis of organics (VOSA, NOVA, and SAM).

(1) Ninety-six narrow mouth, amber glass, sample bottles with Teflon lined screw caps. For drinking and surface water, one gallon bottles calibrated to 3.5 L are used, for all other water types, one quart bottles calibrated to 920 mL are used. Bottles are calibrated by adding an accurately measured volume of water, then marking the level of the water miniscus by scratching the outside surface. Bottles should be calibrated before cleaning.

(2) 10 mL graduated pipettes for adding internal standard solutions.

(3) Pasteur pipettes plus pipette bulbs.

(4) One Bausch and Lomb Mini Spec 20 portable spectrometer.

(5) One 25 mL mixing cylinder (Hach Chlorine Determination Kit).

(6) Materials (e.g., "Blue Ice®") for chilling the sample during shipping and storage; insulated shipping containers for samples.

(7) Volumetric flasks (1000, 500, and 10 mL) for preparing standard solutions.

(8) Graduated cylinders - 1000 mL, 50 mL.

(9) Syringes for preparing standard solutions - 100 and 250 μL.

(10) Graduated pipettes for preparing standard solutions - 1 mL.

(11) Teflon lined screw cap bottles for storing standard solutions.

(12) One hundred 3 dram vials with 15 mm screw caps (Supelco 3-3112) and Teflon lined rubber septa (Supelco 3-3115).

(13) Teflon lined rubber septa (Supelco 3-3115).

(14) Reagents

  (a) reagent water - reagent water is defined as water in which no interferences are observed at the detection limit for the parameters of interest. A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

  (b) 1000 mL internal standard solution (For use if NBS ampoules are not available; Table 5.2). Set 1 standards are used for spiking drinking and surface water samples. Set 2 standards are used for spiking municipal and industrial wastewater effluents and energy effluents. The same internal standard solution is used for VOSA, NOVA, and SAM analyses. Internal standard solutions can be prepared from pure certified standards or certified solutions.

  (c) 10 mL standard solutions (Table 5.3) for VOSA, NOVA, and SAM analyses. Standards are prepared from pure stock solutions.

  (d) sodium hydroxide solution - 0.1N in reagent water (must be demonstrated as contaminant free; Chapter 10, 10.3.6.3, Chapter 11, 11.3.6.3, and Chapter 12, 12.3.6.3).

Table 5.2. INTERNAL STANDARD SOLUTIONS[a]

Compound	Amount: Set 1 (µg/7.5 mL)[b]	Amount: Set 2 (µg/10 mL)[c]
n-Butylamine-d_9	74	370
n-Butyric acid-d_7	14	480
2-Naphthalene sulfonic acid-$d_7 \cdot H_2O$	100	500
N-Ethyl-2-fluorobenzylamine	100[d]	500[d]
2-Phenylethyl-1,1,2,2-d_4-amine	97	480

[a] Prepared in reagent water.

[b] Also, µg spiked into 3.5 L of sample.

[c] Also, µg spiked into 920 mL of sample.

[d] Density unknown - calculated assuming d = 1.

Table 5.3. STANDARD SOLUTIONS

Solutions	Concentration (µg/mL)	Solvent
Low Molecular Weight Volatile Acids (VOSA)		
Acrylic acid	530	acetone
Methylbutyric acid	470	acetone
2-Methylcyclopropane carboxylic acid	510	acetone
2-Hexenoic acid	450	acetone
Octanoic acid		
Nonvolatile Acids (NOVA)		
Benzene sulfonic acid	500	acetone
Nitrobenzoic acid	500	acetone
Pentachlorophenol	500	acetone
Chlorobenzene sulfonic acid	500	acetone
2,4,5-Trichlorophenoxyacetic acid	500	acetone
Amines (SAM)		
Allylamine	380	MBE[a]
Diallylamine	390	MBE
Hexylamine	380	MBE
Benzylamine	490	MBE
Dibenzylamine	510	MBE
2-Methylpiperidine	420	MBE

[a] Methyl-*t*-butyl ether.

(e) sulfuric acid solution - 0.1N in reagent water (must be demonstrated as contaminant free; Chapter 10, 10.3.6.3, Chapter 11, 11.3.6.3, and Chapter 12, 12.3.6.3).

(f) 250 mL $Na_2S_2O_3$ solution - 0.05 M in reagent water (must be demonstrated as contaminant free; Chapter 10, 10.3.6.3, Chapter 11, 11.3.6.3, and Chapter 12, 12.3.6.3).

(g) DPD Total Chlorine Reagent Powder Pillow (Hach Chemical Co.).

(h) methylene chloride - distilled in glass.

(i) acetone - distilled in glass.

(j) methyl t-butyl ether - pesticide quality or equivalent.

(k) NBS ampoules containing VOSA, NOVA and SAM deuterated internal standards in water (Table 5.4); optional.

5.3 PREPARATION OF STANDARD SOLUTIONS

5.3.1 Set 1 Internal Standard Solution - Table 5.2 (For use if NBS ampoules are not available; for drinking and surface waters)

(1) Accurately weight 66.6 mg of 2-naphthalene sulfonic acid-$d_7 \cdot H_2O$, then dissolve in reagent water in a 500 mL volumetric flask. With a 100 µL syringe accurately measure 65 µL of the liquid standards into the same flask (for d_7-butyric acid use 18 µL). To determine the quantity of N-ethyl-2-fluorobenzylamine added to the solution, weigh the dry syringe then weigh the syringe containing neat standard. The difference between these two weights is the weight of material delivered to the solution. The syringe must be rinsed with solvent to assure that all of the neat material is delivered. Dilute the sample to volume with reagent water and mix well.

(2) Transfer this stock solution into a Teflon sealed screw cap bottle. Store at 4°C and protect from light.

(3) Accurately measure 100 mL of this stock and transfer to a 1000 mL volumetric flask. Dilute to volume with reagent water and mix well.

Table 5.4. NBS AMPOULES CONTAINING VOSA, NOVA, AND SAM INTERNAL STANDARDS IN WATER

Compound	Concentration (µg/Ampoule)[a] Set No. 1[b]	Concentration (µg/Ampoule)[a] Set No. 2[c]
n-Butyl-d_9-amine	118.3	554.3
n-Butyric-d_7 acid	15.7	535.9
2-Naphthalene-d_7-sulfonic acid · H_2O	99.4	497.4
N-ethyl-2-fluorobenzylamine	107.2	522.1
2-Phenylethyl-1,1,2,2-d_4-amine	121.2	530.3

[a] Concentration in the "400" series production run; Set No. 1 and Set No. 2 contains 7.5 and 10.0 mL water, respectively.

[b] Also, µg spiked into 3.5 L of sample.

[c] Also, µg spiked into 920 mL of sample.

(4) Transfer 7.5 mL portions of this solution to 3 dram vials. Seal with Teflon lined, septum seal, screw caps. Store at 4°C and protect from light.

(5) Fresh standards must be prepared every six months. If signs of degradation or evaporation occur they should be replaced more frequently.

(6) When compound purity is 96% or greater, the weight (or volume) can be used without correction to calculate concentration. Compounds that are less than 96% pure cannot be used for standards.

5.3.2 Set 2 Internal Standard Solutions - Table 5.2 (For use if NBS ampoules are not available; for industrial and energy wastewater effluents)

(1) Accurately weigh 50 mg of 2-naphthalene sulfonic acid - $d_7 \cdot H_2O$, then dissolve in reagent water in a 1000 mL volumetric flask. With a 100 µL syringe, accurately measure 50 µL of the liquid standards into the same flask. Dilute the sample to volume with reagent water and mix well. To determine the quantity of N-ethyl-2-fluorobenzylamine added to the solution, weigh the dry syringe then weigh the syringe containing the neat compound.

The difference between these two weights is the weight of material delivered to the solution. The syringe must be rinsed well with solvent to assure that all of the neat material is delivered.

(2) Transfer 10 mL portions solution into 3 dram vials. Seal with Teflon lined, septum seal screw caps. Store at 4°C and protect from light.

(3) Prepare fresh standards every six months. If signs of degradation or evaporation occur they should be replaced more frequently.

5.3.3 Standard Solutions (Table 5.3)

(1) Prepare individual stock solutions (50 mg/mL) for each standard compound.

- Solids - accurately weight about 0.500 gram of pure material. Dissolve the material in distilled in glass quality acetone, dilute to volume in a 10 mL volumetric flask.
- Liquids - with a 1 mL graduated pipette. Accurately add 500 µL of standard compound to the specified solvent (Table 5.3) in a 10 mL volumetric flask. Dilute to volume.

(2) Transfer stock solutions into a Teflon sealed screw cap bottle. Store at 4°C and protect from light. Prepare fresh solutions every six months. If signs of degradation or evaporation occur, they should be replaced more frequently.

(3) Prepare three standard solutions for: 1) the low molecular weight carboxylic acids; 2) the nonvolatile organic acids; and 3) the amines as shown in Table 5.3. With a 250 µL syringe, accurately add 100 µL of each stock standard to the specified solvent in a 10 mL volumetric flask and dilute to volume. This gives the concentration in Table 5.3. Transfer to a Teflon sealed screw cap bottle. Store at 4°C and protect from light. Prepare fresh standards every six months. If signs of degradation or evaporation occur, they should be replaced more frequently.

(4) When compound purity is 96% or greater, the weight (or volume) can be used without correction to calculate the concentration of stock standards. Compounds which are less than 96% pure cannot be used for standards.

5.4 GLASS CLEANING PROCEDURES

(1) All glassware to be used, including sample bottles, should be washed with Amway S-A-8 laundry detergent, rinsed several times with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with foil (precleaned with hexane) to prevent contamination.

(2) Teflon liners and Teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. Store in a clean vial.

5.5 PREPARATION OF CONTROL AND BLANK SAMPLES

(1) Dispense aliquots of reagent water into 24 sample bottles. For drinking water, 3.5 L samples will be placed in 1 gallon bottles. For all other water types, 920 mL will be aliquoted into 1 quart bottles. Number the bottles 1 to 24.

(2) Measure total chlorine in the water sample and, if necessary, quench using sodium thiosulfate (Section 6, step 2 and 3).

(3) Spike all samples with internal standard solutions (Table 5.2 or 5.4). If drinking or surface water samples are to be collected spike with 7.5 mL (1 vial) of set 1 standard. If municipal, industrial or energy effluents are to be collected spiked with 10 mL (1 vial) of set 2 standards.

(4) Spike with standard solutsion (Table 5.3) and label sample bottles as described in Table 5.5.

(5) Adjust the sample pH in all bottles as specified in Table 5.5 using sulfuric acid or sodium hydroxide solution. Use narrow range pH paper to measure pH.

(6) Add 2% (v/v) methylene chloride to all samples. Cap bottles and mix well.

(7) Field controls and field blanks are shipped to the collection site and are handled, stored, and shipped in the same manner as field samples. Laboratory blanks and controls are stored at 4°C in the laboratory.

5.6 FIELD COLLECTION

(1) Water samples are collected by rinsing sample bottles three times with sample water, then filling to the calibration mark.

Table 5.5. CONTROL AND BLANK SAMPLES

Bottle	Spike	Handle	Sample pH	Sample Type
1,2	1.0 mL VOSA standard solution	send to field	7-8	field control VOSA
3,4	1.0 mL VOSA standard solution	store in lab	7-8	laboratory control VOSA
5,6	none	send to field	7-8	field blank VOSA
7,8	none	store in lab	7-8	laboratory blank VOSA
9,10	1.0 mL NOVA standard solution	send to field	7-8	field control NOVA[b]
11,12	1.0 mL NOVA standard solution	store in lab	7-8	laboratory control NOVA
13,14	none	send to field	7-8	field blank NOVA
15,16	none	store in lab	7-8	laboratory blank NOVA
17,18	1.0 mL SAM standard solution	send to field	4-5	field control SAM
19,20	1.0 mL SAM standard solution	store in lab	4-5	laboratory control SAM
21,22	none	send to field	4-5	field blank SAM
23,24	none	store in lab	4-5	laboratory blank SAM

[a] Low molecular weight carboxylic acids.

[b] Nonvolatile acids.

(Remember that separate water samples for each of the VOSA, NOVA and SAM protocols must be taken and spiked with internal standards.) Internal standards are added to each water sample. NBS ampoules (Table 5.4) or laboratory prepared standards (Table 5.2) are used. For drinking water and surface waters, 7.5 mL (1 vial) of the Set No. 1 is used. Municipal, industrial, and energy wastewater effluents are spiked with 10 mL (1 vial) of Set No. 2. Rinse the vials containing the internal standard solutions three times with sample water and add to the sample bottle.

(2) A preliminary water sample (~25 mL) is collected for the purpose of determining total chlorine content. This measurement is made at the sample collection site and utilizes the Bausch and Lomb Mini Spec 20 portable spectrophotometer. The water sample is added to a 25 mL mixing cylinder along with the contents of a DPD Total Chlorine Reagent Powder Pillow (Hach Chemical Co.). The mixture is shaken and the color is allowed to develop for 3 to 6 minutes. Percent transmittance, measured at 530 nm, is converted to mg/L total chlorine using Table 5.6.

Table 5.6. TOTAL CHLORINE (mg/L as Cl) VS. % TRANSMITTANCE

%T	% T Units									
Tens	0	1	2	3	4	5	6	7	8	9
10	4.00	3.84	3.68	3.54	3.42	3.30	3.18	3.08	2.98	2.88
20	2.80	2.71	2.63	2.55	2.48	2.41	2.34	2.28	2.21	2.15
30	2.09	2.04	1.98	1.93	1.88	1.82	1.78	1.73	1.68	1.64
40	1.59	1.55	1.51	1.47	1.43	1.39	1.35	1.31	1.28	1.24
50	1.20	1.17	1.14	1.10	1.07	1.04	1.01	0.98	0.95	0.92
60	0.89	0.86	0.83	0.80	0.78	0.75	0.72	0.70	0.67	0.64
70	0.62	0.60	0.57	0.56	0.52	0.50	0.48	0.46	0.43	0.41
80	0.39	0.37	0.34	0.32	0.30	0.28	0.26	0.24	0.22	0.20
90	0.18	0.16	0.14	0.13	0.11	0.09	0.07	0.05	0.04	0.02

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(3) Residual chlorine is quenched by adding a stoichiometric quantity of 0.05 M $Na_2S_2O_3$ (Figure 5.1, a 10% excess is used and is included in calculation) to the bottle containing the aqueous sample.

(4) Samples collected for the analysis of VOSA and NOVA fractions are adjusted to pH 7 to 8. Samples collected for the analysis of SAM are adjusted to pH 4 to 5. Use narrow range pH paper to measure pH. Use sodium hydroxide or sulfuric acid for pH adjustment.

(5) Add 2% (v/v) methylene chloride to all samples as a preservative, cap the samples, and shake well. The samples must be maintained at 4°C from the time of collection until extraction.

(6) Samples should be shipped on ice (e.g., "Blue Ice®") directly to the laboratory by an appropriate air carrier (e.g., Federal Express) in well insulated cartons.

(7) As soon as the samples arrive at the laboratory, the surrogate samples must be spiked with target compounds. Samples for the analyses of VOSA, NOVA, and SAM compounds are spiked with the corresponding standard spiking solution (Table 5.3). Drinking water and surface water samples are spiked with 1.0 mL of the appropriate solution using a graduated pippette. Industrial, municipal and energy wastewaters are spiked with 1 mL of the appropriate solution using a 1 mL graduated pipette.

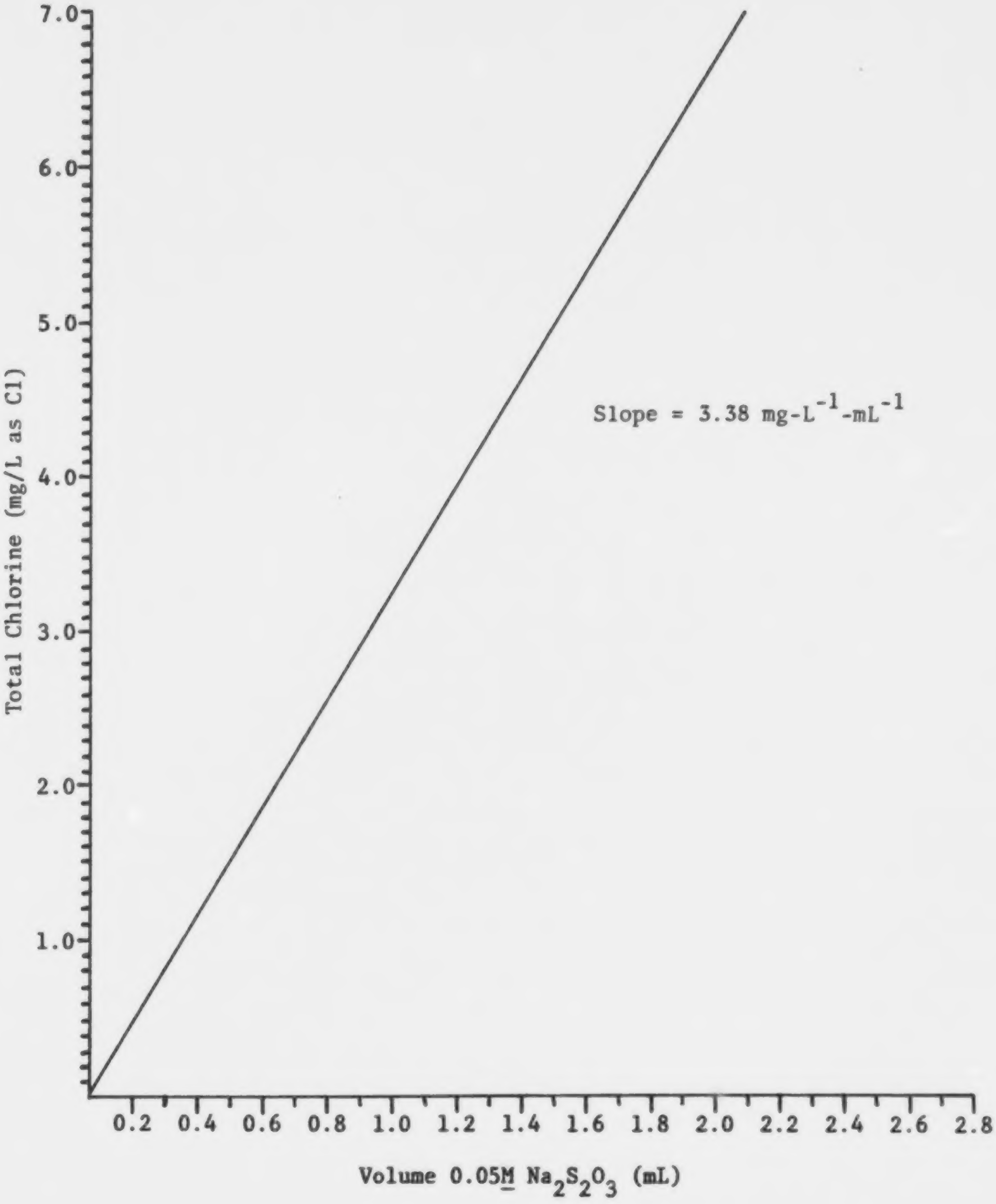


Figure 5.1. Total chlorine vs volume of 0.05M $Na_2S_2O_3$ added to a 1 L sample (if a 3.5 L sample is collected, 3.5x the amount of $Na_2S_2O_3$ must be added).

CHAPTER 6

PURGE, TRAP AND ANALYSIS OF VOLATILE ORGANICS (VO)

6.1 INTRODUCTION

6.1.1 Principle of the Method

This analytical protocol is for the determination of volatile organic (VO) compounds in drinking water, surface waters, and municipal, industrial and energy wastewater effluents. The procedure utilizes helium gas purging through buffered water samples at high ionic strength to effectively partition semisoluble and insoluble volatile organic compounds between the gaseous and aqueous phases, followed by adsorption from the gaseous phase onto a Tenax GC sorbent trap. A custom built purge and trap system is employed in combination with capillary gas chromatography-mass spectrometry. (Fabrication of the system is described in Appendix A; it is also required for the NEWS compounds protocol, see Chapter 7). After trapping on Tenax, the organics are thermally desorbed and sample components are cryofocussed in a liquid nitrogen trap. An external standard (perfluorotoluene) is added, and the VO compounds are flash evaporated onto a capillary column.

6.1.2 Detection Limits and Sample Size

Since the same initial sample size is not used for all water types, detection limits will depend upon the final volume of water sample purged which is determined from a headspace scouting method. The maximum water volume purged is 200 mL; however, dilutions of 1 to 100 may be necessary for some industrial effluents (the purged volume is always 200 mL). If the detection limit for qualitative GC/MS analysis of each VO compound is 10 ng, then the nominal detection limits may range from 0.1 ppb (for 200 mL of drinking water) to 10 ppb, depending upon the dilution factor required. Usually, the limits of detection for quantitative analysis of target compounds are a factor of five lower.

6.1.3 Interferences

Background contamination from impure purging and carrier gases may be experienced if prescribed precautions are not instituted. A hydrocarbon background may occur from the laboratory atmosphere; e.g., from the solvent employed with electrostatic printer/ plotters used with GC/MS systems. The solvent may adsorb on the purging glassware that is stored nearby before use.

Some phenols and amines, because of their polarity, may tail severely during gas chromatographic analyses and mask compounds of interest. If the pH of the sample is adjusted to seven, as directed, the phenolics and amines are mostly in an ionic state and will not purge.

6.1.4 Recoveries and Scope

Table 1.3 (Chapter 1) presents the mean percent recovery and standard deviation for VO compounds in drinking water and an industrial/municipal effluent. Compound classes most amenable to this technique include ethers, aliphatic hydrocarbons, halogenated aliphatic hydrocarbons, aromatic hydrocarbons and halogenated aromatic hydrocarbons. Esters are recovered only moderately and aldehydes and ketones are recovered poorly; volatile compounds in these classes are best handled by the NEWS protocol (Chapter 7). Generally, compounds exhibiting good recoveries ($\geq$60%) are those with boiling points below 200°C (vapor pressure $\simeq$1 mm Hg @ 25°C), and in several instances below 250°C.

6.2 APPARATUS AND REAGENTS (To Process Six Samples)

(1) A custom built purge and trap system (Fig. 6.1, fabrication described in Appendix A).

(2) Six purge flasks, 200 mL capacity (Fig. 6.2), calibrated (Section 6.3.4). Sample transfer system (Fig. 6.3).

(3) Reagents

- (a) Sodium sulfate, anhydrous powder (ACS grade and free of VO compounds, Section 6.3.3), 350 g.
- (b) pH 7.0 Phosphate buffer; 0.95 g K_2HPO_4 + 2.96 g $NaH_2PO_4 \cdot 7 H_2O$ (6 weighed aliquots).
- (c) 1.5 L reagent water (high purity water, purged for 30 min @ 90°C, $\geq$50 mL/min with Ultra-pure Helium gas).

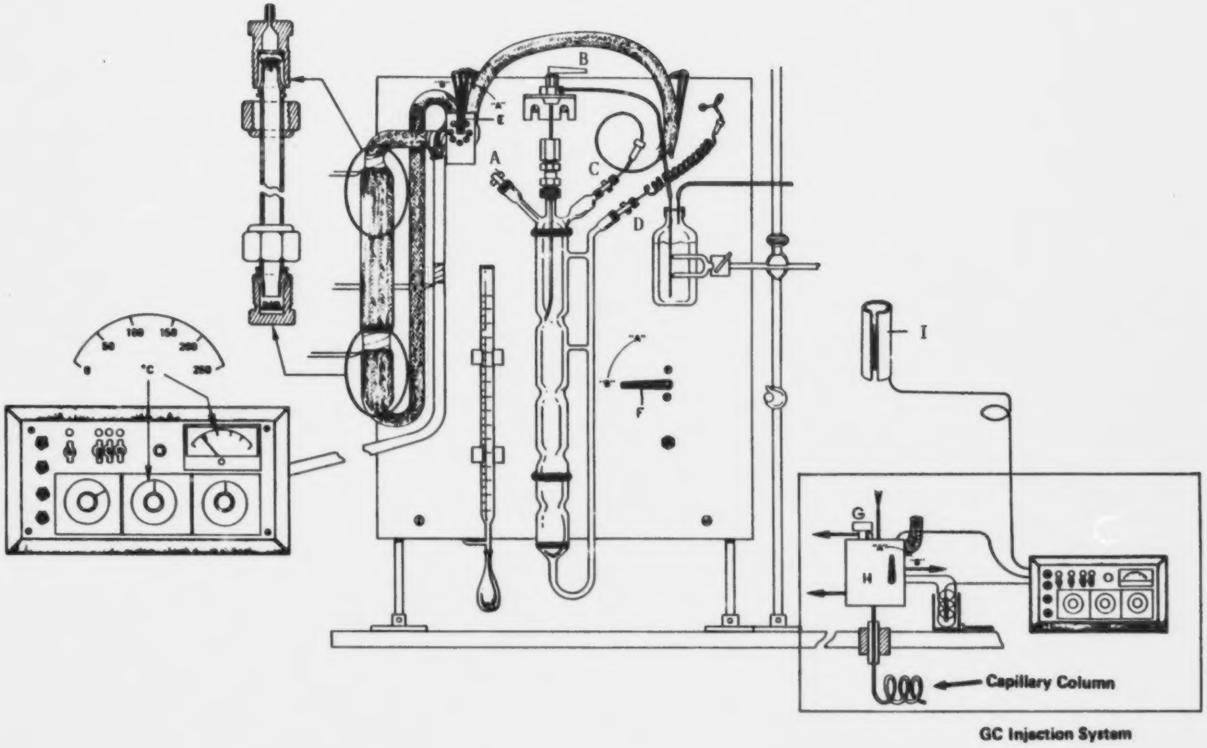

A
B
C
D
E
"A"
"B"
F
G
H
I
0
50
100
150
200
250
°C
Capillary Column
GC Injection System

Figure 6.1. Purge and trap system with injection system (front view).

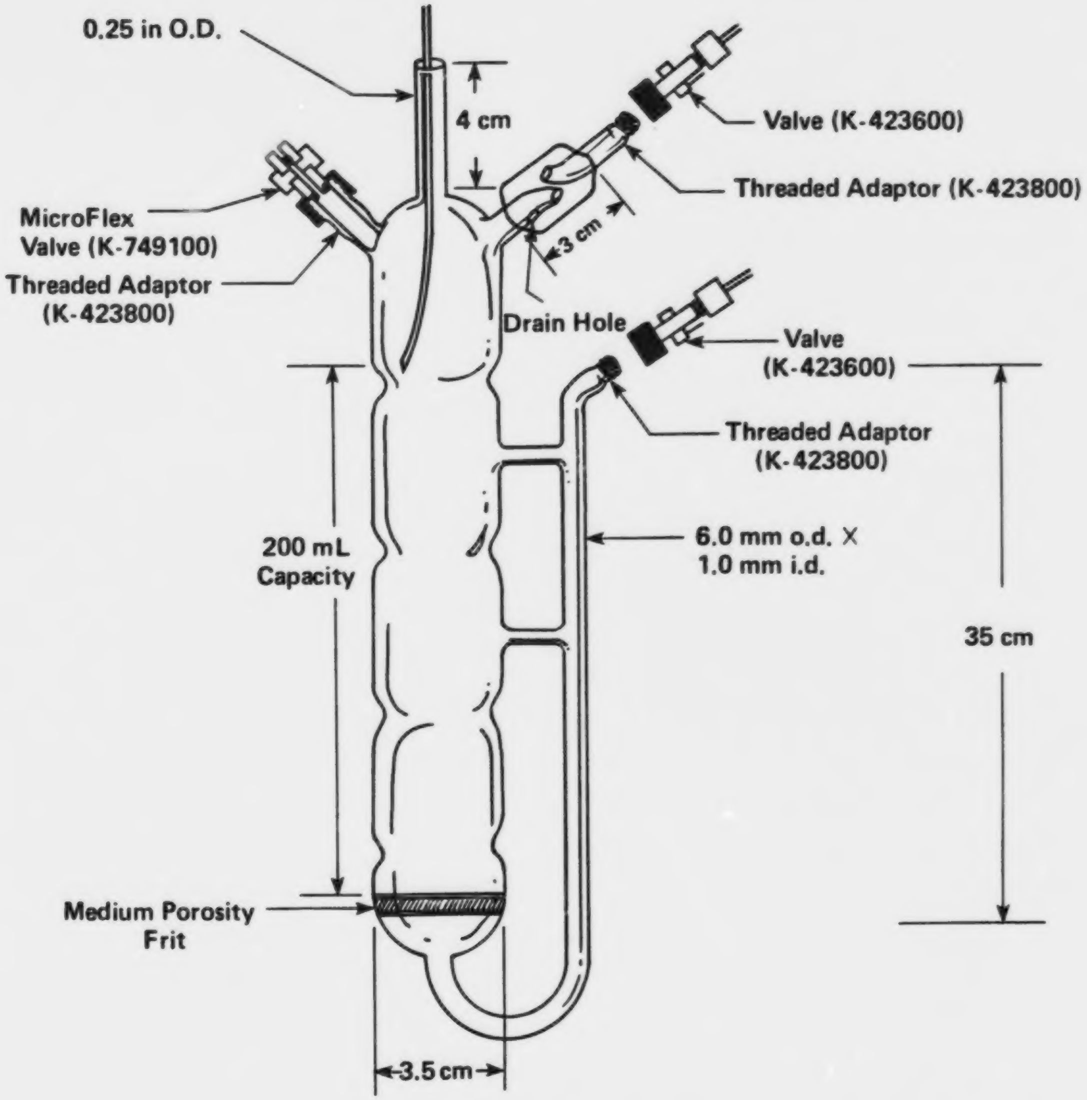

0.25 in O.D.
4 cm
Valve (K-423600)
Threaded Adaptor (K-423800)
MicroFlex
Valve (K-749100)
Threaded Adaptor
(K-423800)
3 cm
Drain Hole
Valve
(K-423600)
Threaded Adaptor
(K-423800)
200 mL
Capacity
6.0 mm o.d. X
1.0 mm i.d.
35 cm
Medium Porosity
Frit
3.5 cm

Figure 6.2. Purge vessel (200 mL capacity).

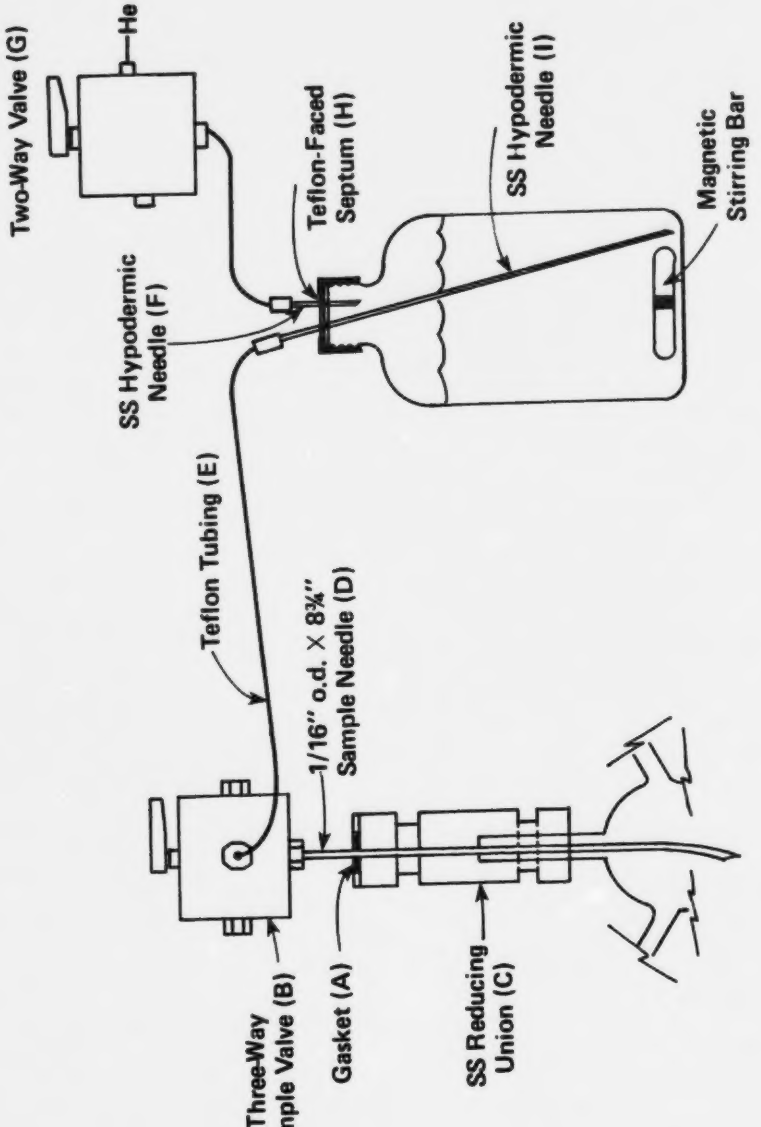

Two-Way Valve (G)
He in
Teflon-Faced Septum (H)
SS Hypodermic Needle (I)
Magnetic Stirring Bar
SS Hypodermic Needle (F)
Teflon Tubing (E)
1/16" o.d. × 8¾" Sample Needle (D)
Three-Way Sample Valve (B)
Gasket (A)
SS Reducing Union (C)

Figure 6.3. Sample transfer schematic.

(d) Ultra-pure Helium gas.

(e) NBS deuterated VO internal standards in glass capsules (see Table 2.2, Chapter 2).

(f) Internal standard solution (optional if NBS capsules are not available; see Table 2.4, Chapter 2).

(g) External standard, perfluorotoluene (50 µL). Acceptable chemical purity is $\geq$98%.

(h) Headspace analysis calibration mixture (Table 6.1).

(i) System performance solution (Table 6.2).

(4) Thermostated water bath. To accommodate two 250 mL bottles at 30-32°C.

(5) Two cryogenic traps (Fig. 6.4): one is for purifying helium to prepare reagent grade water, the second for providing liquid nitrogen to the cryofocussing trap of the injection system. Two 1 L Dewar flasks, copper tubing, SS tubing, pressure relief valve, and flow control valve V (Fig. 6.4).

(6) Syringes:

(a) 1 µL, 5 µL, 10 µL (Hamilton Gas-Tight® or equivalent).

(b) 5 and 10 mL gas sampling syringes with shut-off valve.

Table 6.1. HEADSPACE CALIBRATION MIXTURE

Chemical	b.p. (°C)	Density (@ 20°C)	Volume[a] (µL)
diethyl ether	35	0.714	14
cyclopentane	49	0.746	13
chloroform	61	1.483	7
thiophene	84	1.065	9
_n_-heptane	98	0.684	15
toluene	111	0.867	12
ethyl butyrate	120	0.878	11
phenyl ether	170	1.075	9
1,4-dibromobutane	197	1.789	6
1,2,4,5-tetrachlorobenzene	244	1.858	5

[a] Volume of neat substance added to a 100 mL volumetric flask containing methanol.

Table 6.2. SYSTEM PERFORMANCE SOLUTION FOR VO COMPOUNDS[a]

Chemical	Density (@ 20°C)	Concentration[b] (ng/µL)	Amount Injected (ng)
1-bromo-4-fluorobenzene	1.495	1,490	300
perfluorotoluene	1.770	1,770	350
d_5-bromoethane	1.460	1,460	290
d_5-chlorobenzene	1.106	1,100	220
2,4,6-d_3-anisole	0.996	990	200
d_8-naphthalene	1.025[c]	1,020	200
_n_-octane	0.702	700	140
_n_-decane	0.730	730	150
acetophenone	1.028	1,030	210
1-octanol	0.827	820	160
5-nonanone	0.822	820	160
2,6-dimethylphenol	0.968	970	190
2,6-dimethylaniline	0.972	970	190
ethylbenzene	0.867	860	170
_p_-xylene	0.861	860	170
_n_-nonane	0.718	5,020[d]	1,000
1,3,5-trimethylbenzene	0.865	52[e]	10

[a] See Table 6.6 for the function of each test component in the system performance evaluation.

[b] Solvent is methylene chloride; 25 µL of neat chemical is delivered to a 25 mL volumetric flask containing 25 mL of solvent.

[c] The mass of d_8-naphthalene added is determined by weighing the solid material.

[d] 175 µL is added.

[e] 1.5 µL is added.

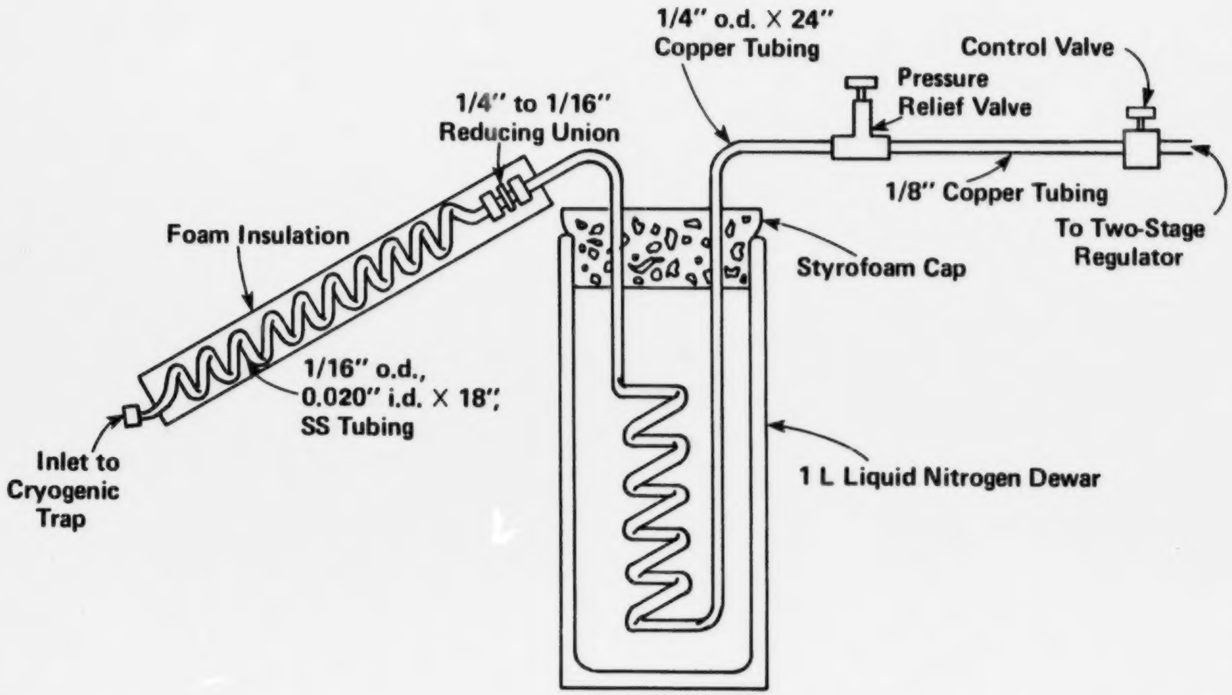

1/4" o.d. X 24"
Copper Tubing
Control Valve
Pressure
Relief Valve
1/4" to 1/16"
Reducing Union
1/8" Copper Tubing
To Two-Stage
Regulator
Foam Insulation
Styrofoam Cap
1/16" o.d.,
0.020" i.d. X 18",
SS Tubing
Inlet to
Cryogenic
Trap
1 L Liquid Nitrogen Dewar

Figure 6.4. Cryogenic system for generating liquid nitrogen from a gas reservoir.

(7) 60 m × 0.32 mm i.d., DB-1 (1.0 µ film thickness) fused silica capillary (or equivalent).

(8) One gas chromatograph (packed column) with flame ionization detector.

(9) 1.8 m × 0.2 cm i.d. glass column packed with 3% OV-17 on 80/100 Gas Chrom Q.

(10) 1 liter glass gas bulb (Fig. 6.5), heating mantle, Teflon® coated magnetic stirbar, and magnetic stirrer.

6.3 PREPARATION FOR ANALYSIS

6.3.1 He Purification

Helium, which is used for preparing reagent grade water, must be passed through a liquid nitrogen-cooled trap to remove volatile components. This trap is constructed of 0.25 in o.d. × 24 in copper tubing, coiled and immersed in a liquid nitrogen Dewar bath (see right side of Fig. 6.4). The copper tubing must be cleaned by rinsing with pentane and air drying under a heat gun prior to use to remove any potential contaminants.

6.3.2 Distilled/Deionized Water Purification

Interfering volatile substances in distilled water and/or deionized water are removed by purging at 90°C with purified helium at >50 mL/min for at least 30 min. Water purified in this manner should be used the same day of its preparation.

6.3.3 Sodium Sulfate Purification

Sodium sulfate purity must be verified prior to use. This is accomplished by purging 60 g of anhydrous Na_2SO_4, contained in the purge and trap vessel, with 500 mL purified helium, and analyzing by GC/MS (Section 4.4). If a significant increase in the number or magnitude of peaks is observed relative to a dry, empty vessel purge, a new lot of Na_2SO_4 must be examined or the existing material must be purified. Sodium sulfate can be purified by heating to 400°C in an oven for 2 hr in a 2 cm diam. × 20 cm quartz tube with a nominal flow of pre-purified nitrogen gas at 20 mL/min. The purified salt should be cooled to approximately 100°C in the quartz tube under N_2 flow, after which it may be transferred to a nitrogen-purged, clean, glass bottle with a Teflon-lined screw cap. Once a satisfactory lot of Na_2SO_4 has been identified, sufficient quantity should be obtained to complete the entire sample batch. Sodium sulfate (anhydrous powder) obtained from J. T. Baker Chemical Co., Phillipsburg, NJ is generally satisfactory for use here, but purity must be verified.

6.3.4 Calibration of Purge Flasks and Gas Bulb

Each purge flask (Fig. 6.2) must be calibrated at a volume equivalent to the total volume of the sample plus the buffer salt and sodium sulfate. To determine this volume, 54 g of anhydrous sodium sulfate powder, 0.95 g K_2HPO_4 and 2.96 g $HaH_2PO_4 \cdot 7H_2O$ are dissolved in 200 mL of water at 30°C. The total volume (~212 mL) is measured with a graduated cylinder. An equivalent volume of water is transferred to each purge flask and a permanent mark is made at the appropriate height on the outside of each purge flask.

The gas bulb in Figure 6.5 is calibrated by filling with reagent water and then measuring the volume of water it held with a graduate cylinder. The gas bulb volume is used to calculate the volume of external standard, perfluorotoluene, to be added to each VO fraction analyzed.

6.3.5 Determination of Dilution Factor Scale

The degree of sample dilution required (if any) to provide successful GC/MS/COMP analysis of the actual samples must be determined relative to the operable dynamic range of the mass spectrometer being employed. This is accomplished by purging reagent water spiked with the components given in Table 6.1. Two standard solutions (0.1 and 1.0 ppm/component) are each prepared in triplicate by injecting 12 µL and 120 µL into each of two 12 mL vials filled (no headspace) with reagent water. An aliquot of each standard solution (4 mL, 2 mL, 1 mL corresponding to 1:50, 1:100, 1:200 dilutions) is transferred to the purge flask containing 200 mL of reagent water and the appropriate salts and analyzed by GC/MS as described under Section 6.4.4.

The GC/MS area counts for principal quantitation ions (as given in Appendix B) are determined (Table 6.3). The dilution factor of each aqueous standard that gives results that are approximately midpoint of the dynamic range of the GC/MS system (e.g., approximately 400 ng of injected analyte) is then indicated as shown, for example, in Figure 6.6 for 0.1 and 1 ppm. For the other VO levels in samples the remainding dilution factors are extrapolated.

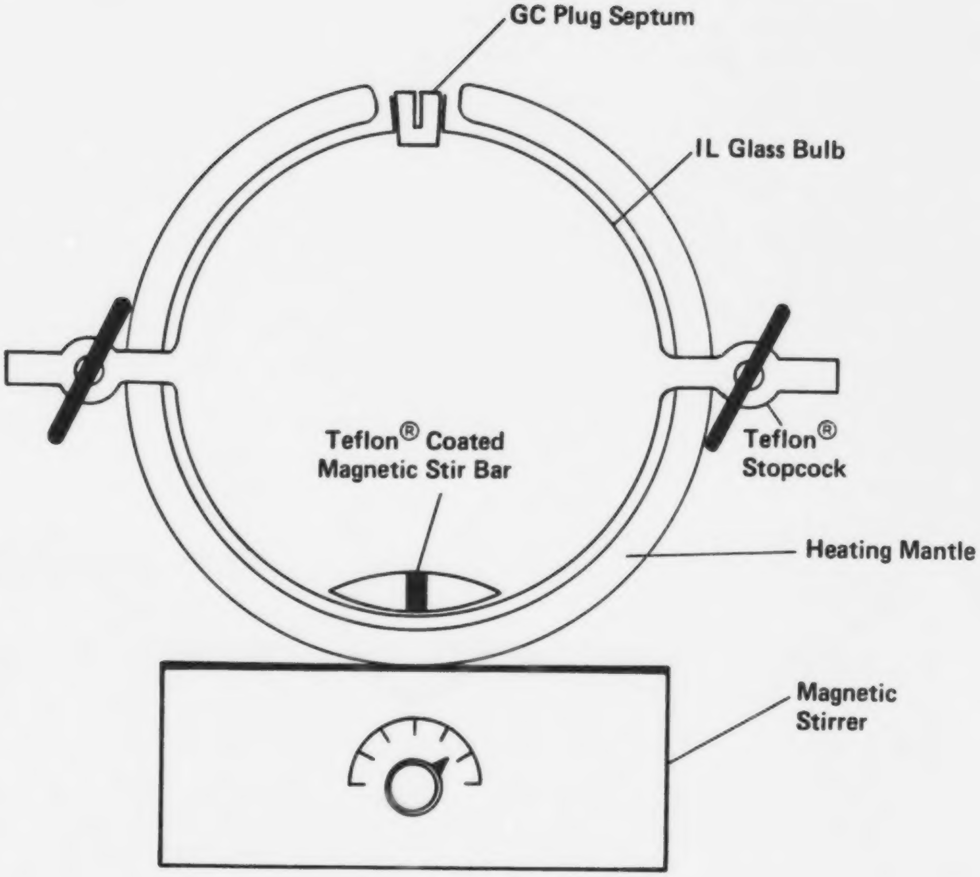

GC Plug Septum
IL Glass Bulb
Teflon® Coated Magnetic Stir Bar
Teflon® Stopcock
Heating Mantle
Magnetic Stirrer

Figure 6.5. Gas bulb and heating mantle for producing gaseous external standard, perfluorotoluene.

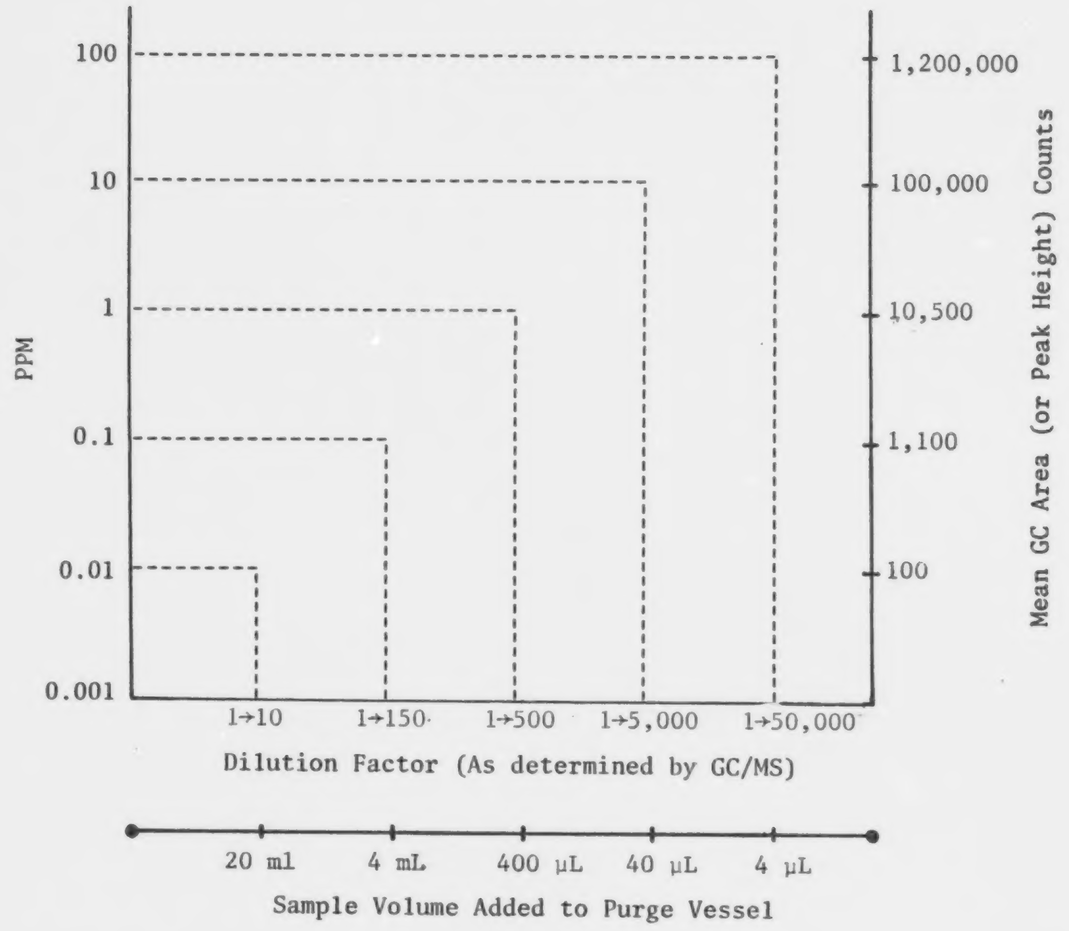


Figure 6.6. Determination of dilution factor for sample analysis from headspace scouting.

Table 6.3. EXAMPLE OF DYNAMIC RANGE RESULTS

Analyte Level Analyzed by GC/MS	Final Dilution Factor	Mean Area Counts For 10 Components
100	1→200	60,000
200	1→100	125,000
400[a]	1→50	245,000
1000	1→200	620,000
2000	1→100	1,250,000
4000	1→50	--[b]

[a] Approximate midpoint of dynamic range of GC/MS system (defined as capillary capacity, but could also be analog/digital interface saturation on some systems).

[b] A/D saturation.

6.3.6 Calibration of GC/FID System for Sample Scouting

The GC/FID system is calibrated for response by analyzing the headspace of four standard solutions of VO compounds (Table 6.1) prepared at 0.01, 0.1, 1 and 10 ppm/component. For example, to prepare a 1 ppm/component mixture, 100 µL of the standard solution (Table 6.1) is injected into a 3 dram (12 mL capacity), septum-capped vial containing 10 mL of 30% aqueous (reagent water) Na_2SO_4 solution. After equilibration at 50°C for 1 hr in a water bath, 10 µL of headspace gas is withdrawn and analyzed by GC/FID on a 1.8 m × 0.2 cm i.d., 3% OV-17 on 80/100 Gas Chrom Q column, temperature programmed from 30° to 200°C at 20°C/min after a 5 min hold at 30°C, with carrier gas flow of 30 mL/min.

The mean of the GC area (or peak height) counts for all peaks are plotted on a graph similar to that shown in Figure 6.6 for each standard solution. Thus, the GC/FID response is equated to the GC/MS response and dilution factor required for successful GC/MS analysis.

6.3.7 Sample Scouting

A 10 mL aliquot of the scouting sample (collected in a 3 dram vials) is transferred to a 3 dram (12 mL), septum-capped vial containing 3 g of pure anhydrous Na_2SO_4. After equilibration at 50°C for 1 hr in a water bath, 100 μL of headspace gas is withdrawn and analyzed by GC/FID on a 1.8 m × 0.2 cm i.d., 3% OV-17 on 80/100 Gas Chrom Q column, temperature programmed from 30° to 200°C at 20°C/min after a 5 min hold at 30°C, with carrier gas flow of 30 mL/min.

The degree of dilution required for the corresponding water samples is then determined from Figure 6.6.

6.4 GC/MS/COMP ANALYSIS

Prior to beginning GC/MS/COMP analysis of VO compounds, the analyst should consult the general procedures described in Chapter 13, entitled "Capillary GC/MS/COMP Analysis Procedure - General Instructions for All Protocols."

6.4.1 Preparation of Gaseous External Standard

A 1 L gas bulb (Fig. 6.5) with a septum port and containing a Teflon coated magnetic stirring bar is flushed for 3 min (~3 L/min) with He, while the gas bulb is in a heating mantle (35°C) positioned on an operating magnetic stirrer. Approximately 0.5-0.7 μL (the exact volume is recorded) of perfluorotoluene (PFT) is injected into the gas bulb with both stopcocks closed and stirred for 30 min to attain complete volatilization and mixing.

6.4.2 GC/MS/COMP Analysis

6.4.2.1 GC/MS/COMP Operating Parameters--

The recommended GC/MS operating parameters are given in Table 6.4 for the analysis of VO compounds. Other capillary columns which meet the system performance criteria may be substituted for the recommended fused silica (See Section 13.4).

6.4.2.2 MS Calibration--

The mass spectrometer is calibrated (Chapter 13) using the manufacturer's recommended approach. The acceptability of the calibration results is checked upon analysis of the system performance solution (SPS).

6.4.2.3 Analysis of SPS (Quality Control)--

Prior to analysis of the SPS or any samples, the sorbent trap (Tenax GC) on the purge and trap system is conditioned at 220°C for 30 min each day when the purge and trap system is used. The 6-port valve (E, Fig. 6.1) should be in position A during conditioning. The temperatures on the system are set as indicated in Table 6.5.

Table 6.4. GC/MS OPERATING CONDITIONS FOR VO COMPOUND ANALYSIS

GC Column	60 m DB-1 wide-bore fused silica capillary column (1.0 μ film thickness)
GC Carrier Gas	Helium
Carrier Gas Flow	1.8 mL/min
Carrier Gas Sweep Rate	28 cm/sec
Temperature Program	35°C for 5 min; 45°C for 5 min; programmed to 240°C at 4°C/min
Injector Temperature	200°C
MS Transfer Line Temperature	260°C
Injection Mode	Splitless
Injection Volume (PFT vapor in helium)	1.0 mL
Ionizing Energy	70 eV
Ion Source Temperature	250°C
Scan Range	35-400
Scan Speed	Scan 0.95 sec, hold 0.05 sec

Table 6.5. TEMPERATURE SET-POINTS ON PURGE AND TRAP SYSTEM (FIG. 6.1)

Parameter	Temperature (°C)
• 6-port valve, E (8)[a]	160
• transfer line (20) from purge flask exit to 6-port valve	160
• transfer lines (21 and 22) from 6-port valve to sorbent trap	160
• transfer line (5) from 6-port to injection system	160
• injection port and valve, G and H (1)	200
• cryofocussing trap (2)	
- bakeout	240
- operational modes	
* cryogenic	-195
* sample injection (flash evaporation)	220
• Tenax GC trap (4)	
- bakeout	220
- sample adsorption	ambient (~23)
- sample desorption	200

[a] ( ) denotes the item numbered in Fig. A-7 and Table A-2 or A-3 of Appendix A.

The system performance mixture (Table 6.2) is analyzed each day before any samples are run. A 0.2 µL injection is made directly into the injection port (G, Fig. 6.1; valve in position "B") with the cryogenic trap cooled to liquid nitrogen temperatures. After 4 min the liquid nitrogen cooling of the cryofocussing trap is discontinued, the trap temperature is raised to 220°C and data acquisition is started.

The SPS raw data are extracted from the run, test parameters calculated and plotted or tabulated (Tables 6.6 and 6.7). Up to 14 days of SPS analytical results can be historically recorded in Table 6.6. This allows the analyst to follow subtle trends which may develop, and anticipate GC or MS maintenance required. Calculation of test parameters and discussion of their use is given in Chapter 13.

A check of RMRs on a daily basis is also calculated from SPS raw data and tabulated (Table 6.7) and compared to that set developed for the historical data bank. The usage of this data is also discussed in Chapter 13.

6.4.3 Determination of RMRs

RMRs for the User's data bank (as opposed to the daily RMR checks discussed in the above paragraph) are determined by injecting either a gaseous mixture (of deuterated internal standards, PFT, and analytes eluting prior to anisole) or a methylene chloride solution (of deuterated internal standards, PFT, and analytes eluting after chlorobenzene) into the injection port of the purge and trap system (G, Fig. 6.1).

6.4.3.1 Gaseous Mixtures of Analytes and Deuterated Internal Standards--

A gaseous mixture is prepared for VO compounds in a similar manner to the gaseous PFT procedure (Section 6.4.1). The amount of compound/mL of gas is calculated from the neat volume injected (converted to mass based on its density) and the volume of the bulb.

6.4.3.2 Solution of Analytes and Deuterated Internal Standards--

This solution is prepared in the same manner as the SPS (Table 6.2).

6.4.3.3 GC/MS/COMP Analysis for RMR Determination--

The standard mixtures of analytes and internal and external standards are injected into the injection port and trapped in the cryofocussing trap

Table 6.6. GC-MS SYSTEM PERFORMANCE TEST FOR VOLATILE ORGANICS (VO)

Dates:				**Run Id Code**		

GC Column and Program: ____________________

Notebook References: ____________________

MS SYSTEM CHECKS

High/Low Mass Balance

Perfluorotoluene:

m/z	69	74	79	93	98	108	111	148	167	186	217	218	236	237
Abundance*	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )
Day														

Bromofluorobenzene:

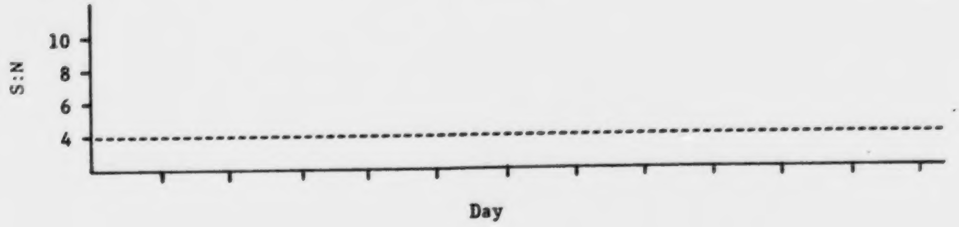
Ion	Relative Abundance Criteria	Abundance Observed Day					
50	15 to 40% of m/z 95						
75	30 to 60% of m/z 95						
95	100%						
96	5 to 9% of m/z 95						
173	<2% of m/z 174						
174	>50% of m/z 95						
175	5 to 9% of m/z 174						
176	>95%, <101% of m/z 174						
177	5 to 9% of m/z 176						

*These abundances are initially established by the MAS user and subsequently become his guideline for acceptability of calibration.

(continued)

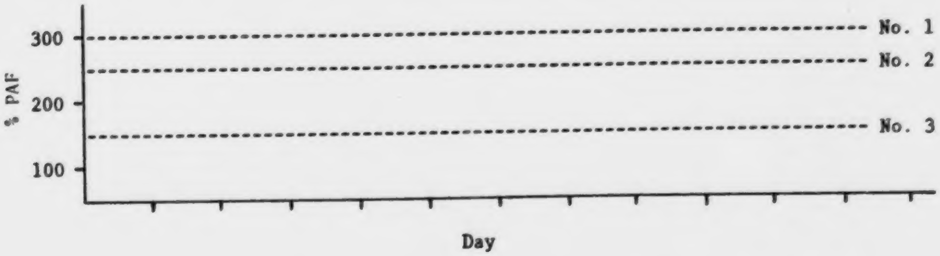
Table 6.6 (cont'd.)

Test	Components	Criteria
Limit of Detection	Trimethylbenzene	S:N (m/z 51) > 4:1

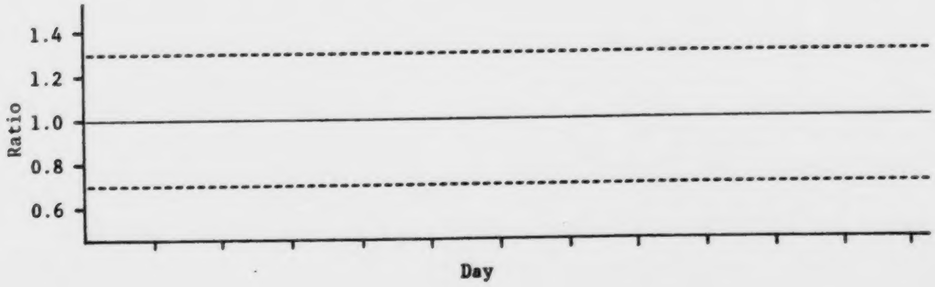


GC SYSTEM CHECKS

Test	Components	Criteria
Peak Asymmetry	1. Acetophenone (m/z 120 or 105) ● 2. 1-Octanol (m/z 70 or 84) ▲ 3. 5-Nonanone (m/z 57) ■	% PAF < 300 % PAF < 250 % PAF < 160



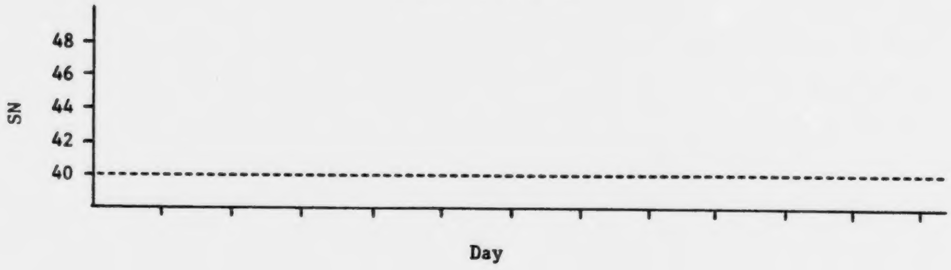
Test	Components	Criteria
Acidity/ Basicity	1. Acetophenone (TIC) 2. 2,6-Dimethylphenol (TIC) 3. 2,6-Dimethylaniline (TIC)	Ratio 2:1 = 0.7 to 1.3 ● Ratio 3:1 = 0.7 to 1.3 ■



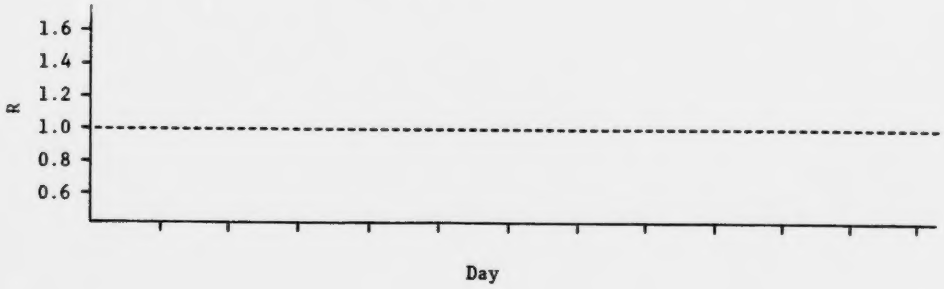
(continued)

Table 6.6 (cont'd.)

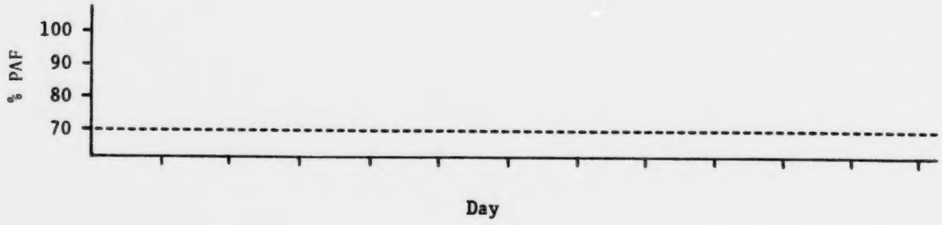
Test	Components	Criteria
Separation Number	n-Octane (m/z 43) n-Decane (m/z 43)	SN $\geq$ 40



Resolution	Ethylbenzene (m/z 91) p-Xylene (m/z 91)	R $\geq$ 1.0



Capillary Capacity	n-Nonane (m/z 43)	% PAF > 70



COMMENTS:

Table 6.7. GC-MS SYSTEM PERFORMANCE TEST-MAS: RMR CHECK FOR VOLATILE ORGANICS (VO)

Date: ____________________

Run ID Code: ____________________

DATA

Standard	MW	Amount (ng)	μM	Ion (m/z)	Area (Run 1)	Area (Run 2)
Perfluorotoluene (PFT)	236			186		
				236		
d_5-Bromoethane	114			113		
				115		
d_5-Chlorobenzene	118			82		
				117		
				119		
d_3-Anisole	111			82		
				111		
d_8-Naphthalene	136			136		

MATRIX OF STANDARD ION RMRs

Standard	Ion	186	236	113	115	82	117	119	82	111	136
PFT	186	-									
	236		-								
d_5-Bromoethane	113			-							
	115				-						
d_5-Chlorobenzene	82					-					
	117						-				
	119							-			
d_3-Anisole	82								-		
	111									-	
d_8-Naphthalene	136										-

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

(Section 6.4.4). A 1 mL volume of the gaseous mixture is used. A 0.2 µL sample of the liquid solution is injected in an identical manner to the introduction of the SPS (Section 6.4.2.3).

The GC/MS/COMP operating parameters were given in Table 6.4. Table 13.8 may be used to tabulate RMR raw data.

6.4.4 Analysis of Blanks, Controls, Samples and Surrogates

A water sample (243 mL bottle) to be analyzed by this purge and trap method is equilibrated at 30-32°C in a thermostated water bath for 20 min.

A clean 200 mL calibrated purge flask is filled with 54 g of Na_2SO_4 and prepurged with purified helium at 25 mL/min for 5 min with valves A and B closed, C disconnected, and D connected and open (Fig. 6.1). Then, valves B and C are opened and C connected via the Teflon line to the purge flask; valves A and D are meanwhile closed during the transfer step (Fig. 6.1). For drinking water, 200 mL is delivered to the purge and trap vessel (to the calibration mark, Section 6.3.4) by pressurizing the sample vessel (Fig. 6.3) using a pressure which transfers the sample in ~4-5 min. The long and short stainless steel needles are inserted respectively through the Teflon faced septum (H) just prior to transfer of the samples. The purge vessel is vigorously shaken (in place) as the sample is introduced and until the Na_2SO_4 is completely dissolved. (Valve C is open so that compounds that volatilize into the purge vessel's headspace pass to the Tenax GC trap which is at ambient temperature during the purging step). Close valve B and the two-way pressurizing valve (G, Fig. 6.3). The water sample is then purged with helium at 25 mL/min for 20 min with valves C and D open (Fig. 6.1). The 6-port (E) and the purge gas valves (F) are in the "A" positions during purging. All valves on the purge vessel are then closed for a "dry" purge step. A 10 min "dry" purge (25 mL/min) to remove excess water from the Tenax GC trap is performed with the 6-port valve (E) in the "A" position and the purge gas valve (F) in the "B" position.

A 1.0 mL gas sample of PFT is injected into the injection port (G, with valve H in "B" position, Fig. 6.1) to introduce the external standard into the cryofocussing trap (which has been cooled to liquid nitrogen temperature), 6 min after "dry" purging has commenced.

Several points need to be emphasized for obtaining reproducibility of PFT measurements by GC/MS:

(1) The needle on the gas sampling syringe (10 mL) should have a rounded solid tip with a side port for gas entrance and exit. (The large gauge of a regular tapered needle point cuts slices out of septa.)

(2) The septum on the injection port must be tight to remain leak-free. A decrease in column head-pressure can be observed on the gauge during injection if the septum is not very tight, even when the side-port needle is used.

(3) Any septum-sweep flow in the injection port must be closed off. As much as 30% of the 1 mL injected can be lost out the septum purge vent.

(4) The Teflon plunger and/or surfaces in the syringe can retain about 10% of the previous PFT analyte. That is, if 1 mL PFT vapor gives a GC area of 100,000 counts, a repeat 1 mL injection of air will yield about 10,000 counts of PFT. If the syringe plunger is pulled up to 2 mL and depressed to 0 mL 5-6 times after PFT injection the area count on a subsequent 1 mL injection of air is less than 2,000 counts.

(5) The syringe should have a closure such that the 1 mL PFT vapor sample can be locked in the syringe and the sample pressurized before injection into the GC; i.e., the plunger at 1 mL graduation is depressed to 0.7 mL before the needle is inserted into the injection port to equalize pressure in the syringe and injection port. (Follow syringe manufacturer's instruction.)

By observing these precautions, the precision of PFT GC/MS response can be maintained at less than 10% relative standard deviation throughout an 8 hr period. However, the PFT gas should be produced fresh each day.

At the end of the "dry" purge step the 6-port valve (E) is rotated to the "B" position (desorb mode), and the injection port valve (H) to the "A" position. The Tenax GC trap is desorbed by heating to 200°C with the He flow at 15 mL/min. After 8 min, the liquid nitrogen cooling of the cryofocussing trap is terminated, the injection port valve (H) is rotated to the "B" position, the temperature on the cryofocussing trap is raised to 220°C, data acquisition started and the GC program conditions are initiated (Table 6.4).

The purge vessel is removed and thoroughly rinsed with deionized water, using suction to pull water through the frit in order to remove residual salt before caking ensues.

For water samples that require dilution prior to purging (as indicated by headspace scouting, Section 6.3.7), the purge vessel is charged later with Na_2SO_4 and buffer salts. Reagent water is added so that the addition of the water sample will brings the final volume to 212 mL (calibration mark). The vessel is shaken to dissolve the salts, with the valves closed. The appropriate water sample volume is withdrawn from the sample bottle into a glass syringe (10 µL, 100 µL, 1 mL or 10 mL, depending on dilution desired) and then injected through valve A of the purge vessel. Valves B and D are closed during addition of the sample aliquot, valve C is open. Purging of the water sample is identical as described above.

6.4.5 Sample Data Analysis

Qualitative and quantitative procedures are described in Chapter 13. Table 6.8 may be used to tabulate sample analyte raw data, prior to using the MASQUANT software program or manual quantitation.

Table 6.8. RAW ANALYTE DATA FOR VO FRACTION

Date: ____________ Run I.D. Code: ____________

Sample Identification: ______ Notebook Reference: ______

Vol. Water (L): ________ Vol. Water Processed (mL): ____
(to which iS were added)

STANDARDS

	I.D. No.	Spectrum No.	Weight (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
Perfluorotoluene				186,236:	,		,
d_5-Bromoethane				113,115:	,		,
2,4,6-d_3-Anisole				81,111:	,		,
d_5-Chlorobenzene				82,117:	,		,
d_8-Naphthalene				136:	,		,

ANALYTES

Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Area	MW_x^a
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	

---

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{RMR_{x/y}} \times \frac{ng_y}{A_y \cdot MW_y}$$

$$K = \frac{ng_y}{A_y \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

CHAPTER 7

ELEVATED TEMPERATURE PURGE, TRAP, AND ANALYSIS OF NEUTRAL WATER SOLUBLE ORGANICS (NEWS)

7.1 INTRODUCTION

7.1.1 Principle of the Method

This analytical protocol is for the determination of volatile low molecular weight, water soluble compounds in drinking water, surface waters, and municipal, industrial and energy wastewater effluents. The procedure utilizes a prepurge of a 10 mL sample with helium at room temperature (to remove VO compounds) and then gas purging while heating (80°C) the water sample (containing 20% NaCl to yield high ionic strength) with adsorption of the NEWS compounds on a Tenax GC sorbent trap. A custom built purge and trap system is employed in combination with capillary gas chromatography/mass spectrometry. (Fabrication of the system is described in Appendix A; it is the same system used for analysis of VO compounds, see Chapter 6.) This approach is used for all water types except drinking water. For drinking water, lower limits of detection are achieved by concentrating a 200 mL sample to 3 mL using azeotropic distillation in a Peter's (Peters, T. I., Anal. Chem., 52, 211, 1980) apparatus. This condensate (5 mL after rinsing the apparatus) is transferred to the purge vessel, made to 20% in NaCl, and then purged at 80°C. In either case, the organics are thermally desorbed from the Tenax, the sample components are cryofocussed, an external standard (perfluorotoluene) is added, and then the NEWS compounds are flash evaporated onto a capillary column.

7.1.2 Detection Limits and Sample Size

If the detection limit for qualitative GC/MS analysis of each NEWS compound is 10 ng, then the nominal detection limits for drinking water and all other water types will be 0.05 and 1 ppb, respectively. Usually, the quantitative limits of detection for target quantitative analysis of compounds are lower.

7.1.3 Interferences

Background contamination from impure purging and carrier gases may be experienced if prescribed precautions are not instituted.

7.1.4 Recoveries and Scope

Table 1.4 (Chapter 1) presents the mean percent recovery and standard deviation for NEWS compounds in drinking water and an effluent. Compound classes most amenable to this technique include aldehydes, ketones, nitriles, alcohols, ethers and nitro containing substances. Esters are not recovered well from the azeotropic distillation step and thus cannot be quantitatively analyzed in drinking water.

7.2 APPARATUS AND REAGENTS (To Process Six Samples)

(1) A custom built purge and trap system (Fig. A-1, fabrication described in Appendix A).

(2) Six purge flasks, 10 mL capacity (Fig. 7.2).

(3) Peter's Apparatus (Fig. 7.3), Vigreau column, 500 mL round bottom flask and heating mantle. The Peter's device is not commercially available and must be fabricated by a glassblower.

(4) Reagents

- (a) NaCl (ACS grade and free of NEWS compounds), 15 g.
- (b) 1.5 L reagent water (high purity water, e.g., Milli-Super Q® water, purged for 1 hr @ 90°C, $\geq$50 mL/min).
- (c) Ultra-pure Helium gas.
- (d) NBS deuterated NEWS internal standards in water (see Table 3.4, Chapter 3).
- (e) Internal standard solution (optional if NBS standards are not available; see Table 3.4, Chapter 3).
- (f) External standard, perfluorotoluene (50 μL). The chemical purity should be $\geq$98%.
- (g) System performance solution (Table 7.1).
- (h) RMR solution.
- (i) 0.006N phosphate buffer (pH 7.0).

(5) Two cryogenic traps: one is used to purify helium for preparation of reagent grade water, the second for providing liquid nitrogen to the cryofocussing module of the injection system

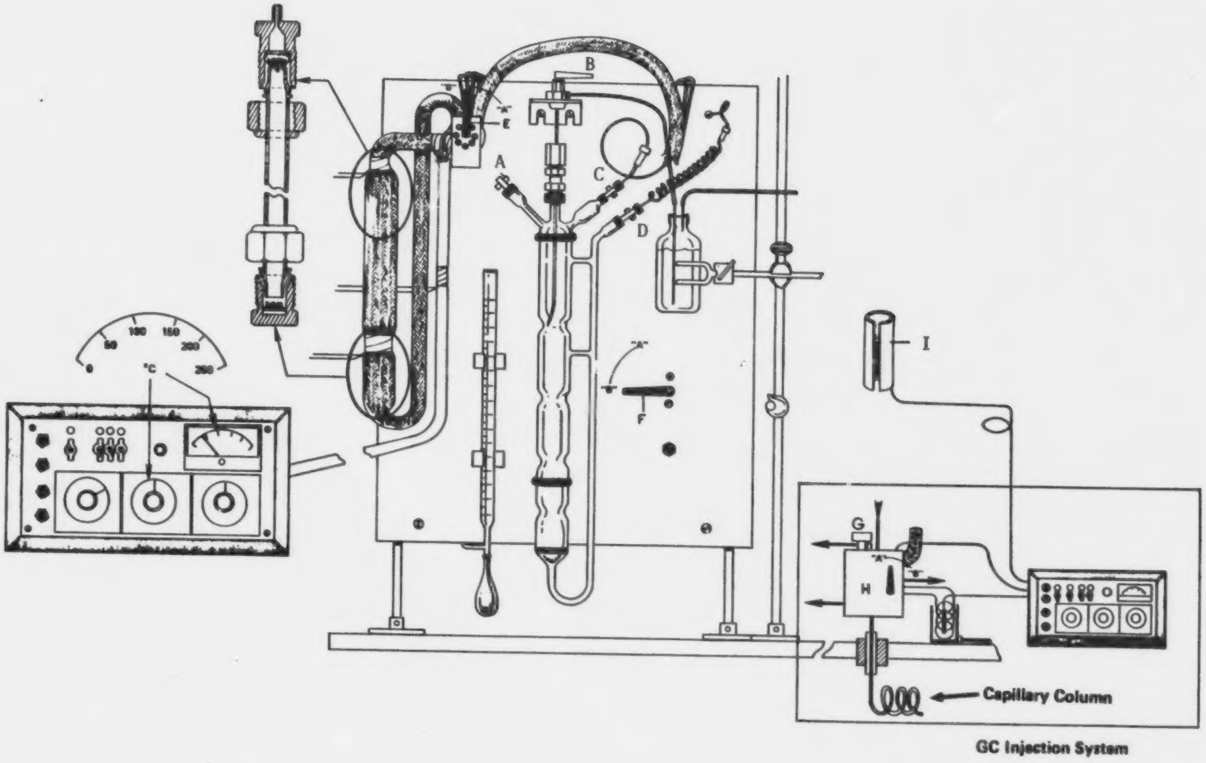

B
"B"
"A"
E
A
C
D
0
50
100
150
200
250
°C
"A"
"B"
F
I
G
"A"
"B"
H
Capillary Column
GC Injection System

Figure 7.1. Purge and trap system (front view).

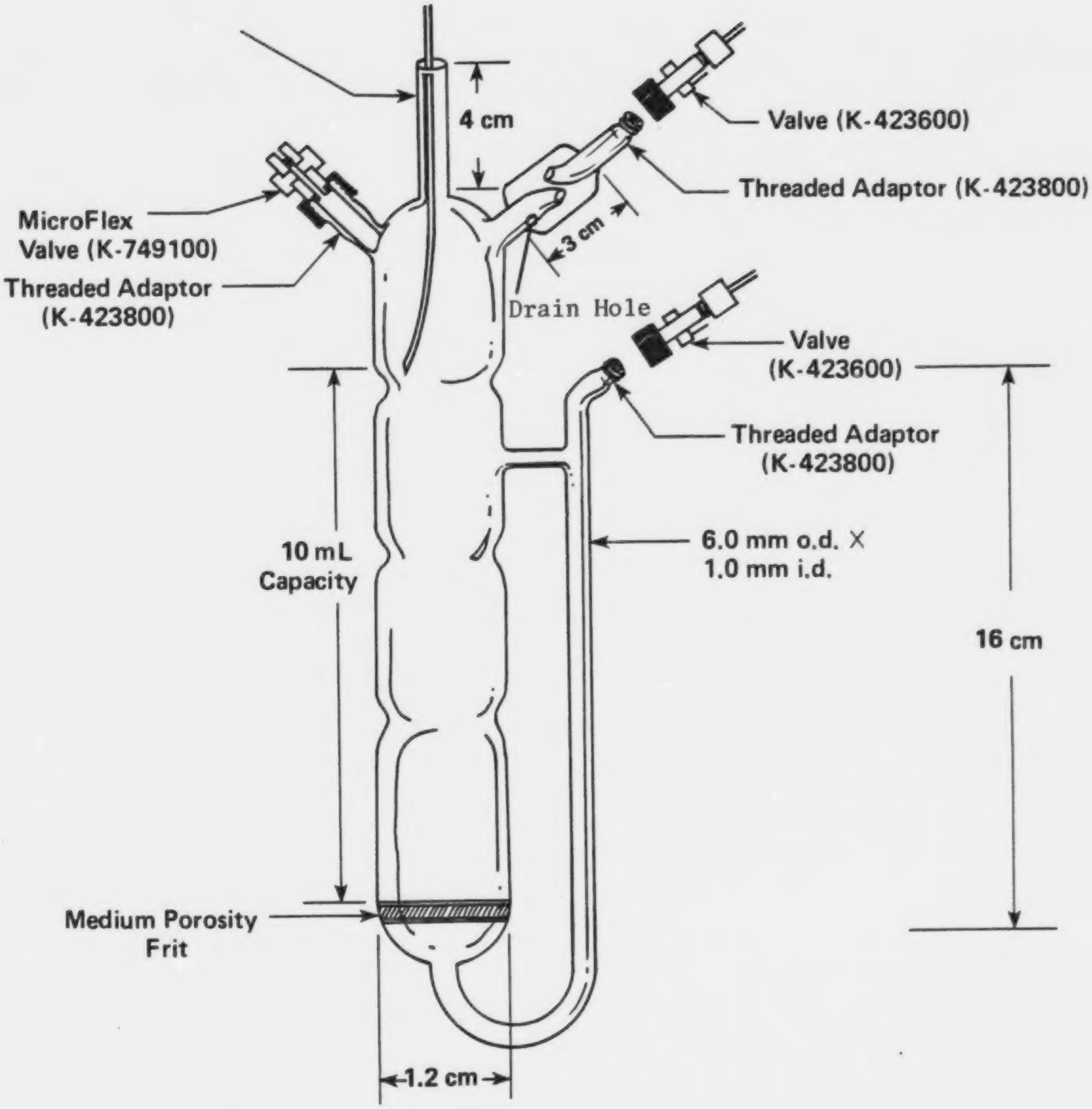

4 cm
Valve (K-423600)
Threaded Adaptor (K-423800)
MicroFlex
Valve (K-749100)
Threaded Adaptor
(K-423800)
3 cm
Drain Hole
Valve
(K-423600)
Threaded Adaptor
(K-423800)
10 mL
Capacity
6.0 mm o.d. X
1.0 mm i.d.
16 cm
Medium Porosity
Frit
1.2 cm

Figure 7.2. Purge vessel (10 mL capacity).

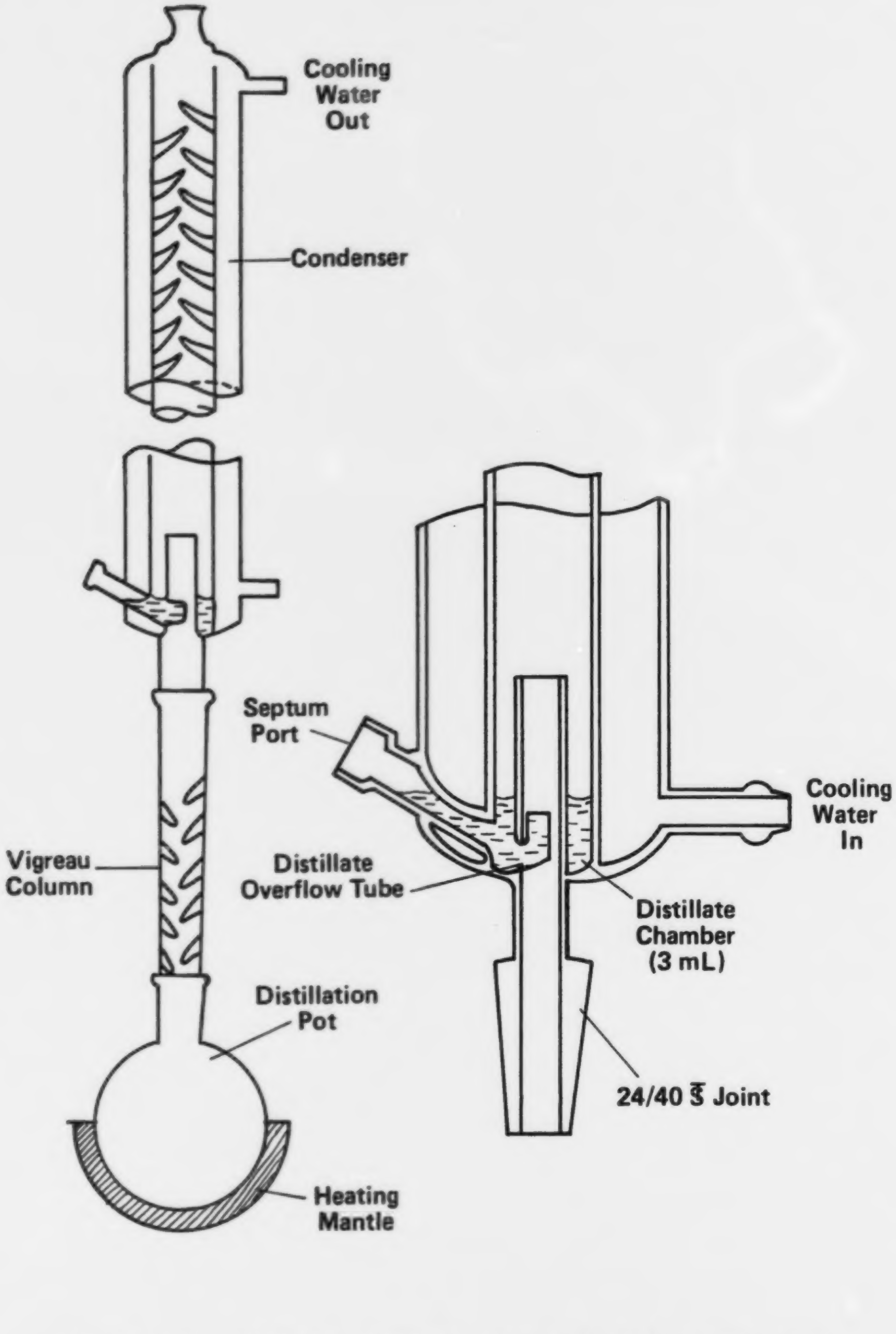

Cooling Water Out
Condenser
Septum Port
Cooling Water In
Vigreau Column
Distillate Overflow Tube
Distillate Chamber (3 mL)
Distillation Pot
24/40 $ Joint
Heating Mantle

Figure 7.3. Peter's concentration-collection apparatus.

Table 7.1. SYSTEM PERFORMANCE SOLUTION FOR NEWS COMPOUNDS[a]

Chemical	Density (@ 20°C)	Concentration[b] (ng/μL)	Amount Injected (ng/μL)
1-bromo-4-fluorobenzene	1.495	1,490	300
perfluorotoluene	1.770	1,770	350
d_9-t-butanol	-	1,500[c]	300
d_5-nitrobenzene	-	1,500[c]	300
n-octane	0.702	700	140
n-decane	0.730	730	150
acetophenone	1.028	1,030	210
1-octanol	0.827	820	160
5-nonanone	0.822	820	160
2,6-dimethylphenol	0.968	970	190
2,6-dimethylaniline	0.972	970	190
ethylbenzene	0.867	860	170
p-xylene	0.861	860	170
n-nonane	0.718	5,020[d]	1,000
1,3,5-trimethylbenzene	0.865	52[e]	10

[a] See Table 7.4 for the function of each test component in the system performance solution.

[b] Solvent is methylene chloride; 25 μL of neat chemical is delivered to a 25 mL volumetric flask containing 25 mL of solvent.

[c] The mass of external and internal standards added is determined by weighing the 10 μL syringe.

[d] 175 μL is added.

[e] 1.5 μL is added.

(Fig. 7.4). Two 1 L Dewar flasks, 0.25 in O.D. copper tubing, 0.125 in I.D. SS tubing.

(6) Syringes:

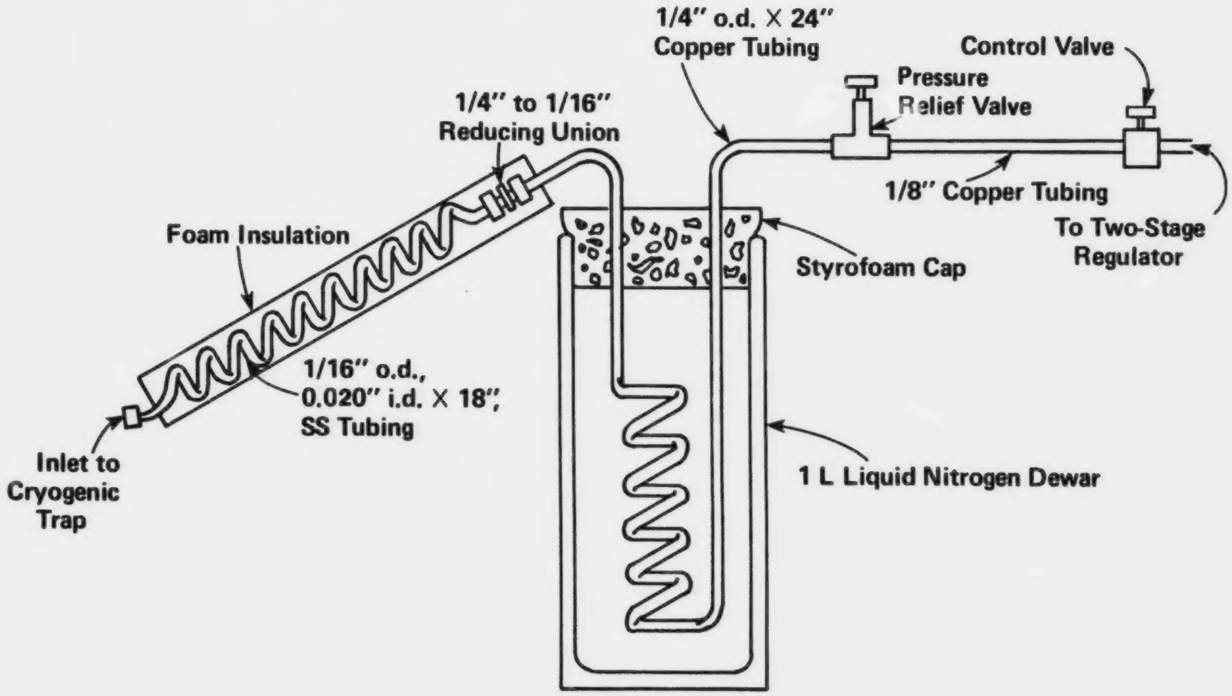

1/4" o.d. X 24"
Copper Tubing
Control Valve
Pressure
Relief Valve
1/4" to 1/16"
Reducing Union
1/8" Copper Tubing
To Two-Stage
Regulator
Foam Insulation
Styrofoam Cap
1/16" o.d.,
0.020" i.d. X 18",
SS Tubing
Inlet to
Cryogenic
Trap
1 L Liquid Nitrogen Dewar

Figure 7.4. Cryogenic system for generating liquid nitrogen from a gas reservoir.

    - (a) 1 μL, 10 μL, 50 μL, 250 μL (Hamilton Gas-Tight® or equivalent).
    - (b) Two 10 mL and one 5 mL gas sampling syringes with shut-off valve.
- (7) 60 m × 0.32 mm i.d., DB-5 fused silica capillary (or equivalent). 1.0 μ film thickness.
- (8) 1 liter glass gas bulb (Fig. 7.5), heating mantle, Teflon® coated magnetic stirbar, and magnetic stirrer.
- (9) Five 5 mL glass ampoules, one Teflon® lined screw cap vial.

7.3 PREPARATION FOR ANALYSIS

7.3.1 He Purification

See Section 6.3.1, Chapter 6.

7.3.2 Distilled/Deionized Water Purification

Interfering volatile substances in distilled water and/or deionized water are removed by purging at 95°C with purified helium at ≥50 mL/min for 1 hr.

7.3.3 Preparation of System Performance Standard (Table 7.1)

With a 50 μL syringe, accurately measure 25 μL of pure liquids into a 25 mL volumetric flask containing about 24 mL of methylene chloride (inject beneath the liquid surface). For the deuterated standards (liquids), weigh the syringe after filling and after delivering the external standard to the volumetric flask. Determine the weight added by difference. For 13,5,-trimethylbenzene, add 1.5 μL. With a 250 μL syringe add 175 μL of *n*-nonane. Make the volume to 25 mL with methylene chloride. Transfer into a series of 2 mL Teflon® sealed screw cap vials. Store at 4°C.

If compound purity is 96% or greater, the weight can be used without correction to calculate the concentration of stock standards. If the compound is less than 96% pure, then it cannot be used as a standard.

7.3.4 Preparation of RMR Solution

Accurately measure 5 μL of neat NEWS compound (those of interest to you) using a 10 μL syringe and inject into a 25 mL volumetric flask containing 24 mL of reagent water. Also, weigh an equivalent amount of d_9-*t*-butanol and d_5-nitrobenzene in a syringe and transfer to the flask. Make the volume to 25 mL. Purity criteria are the same as for the SPS (Section 7.3.3 above).

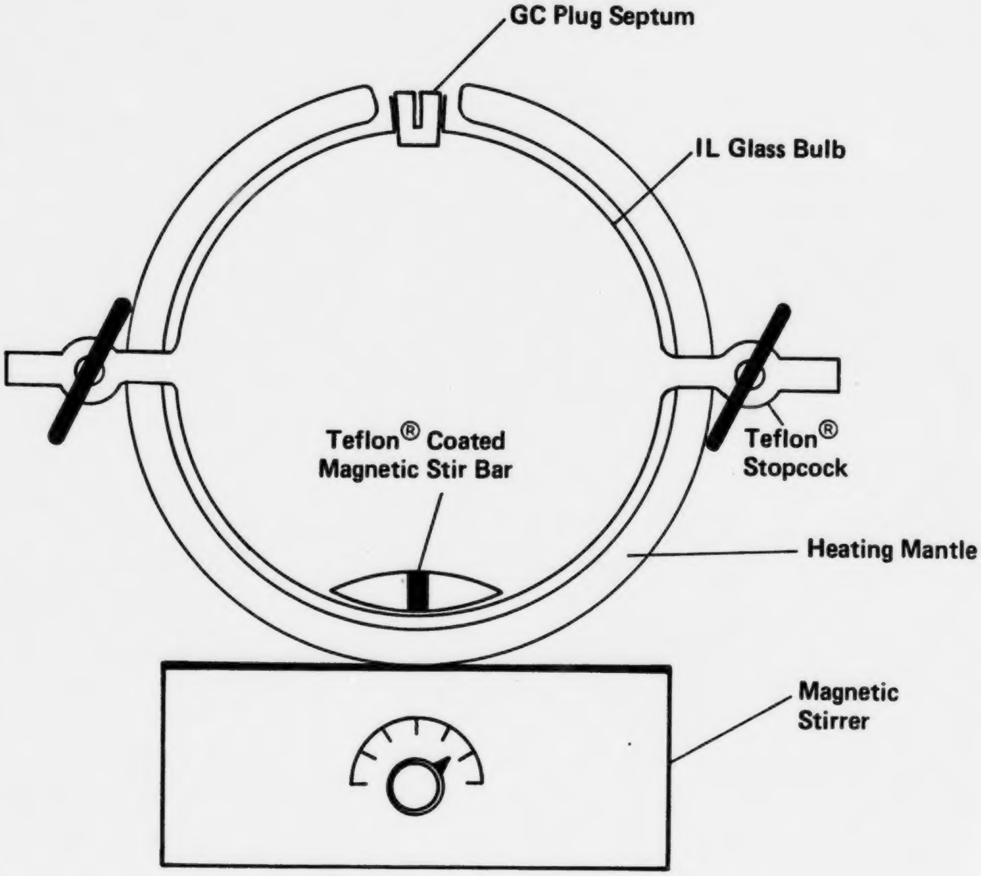

GC Plug Septum
IL Glass Bulb
Teflon® Coated Magnetic Stir Bar
Teflon® Stopcock
Heating Mantle
Magnetic Stirrer

Figure 7.5. Gas bulb and heating mantle for producing gaseous external standard, perfluorotoluene.

Transfer aliquots of the RMR solution into 5 mL glass ampoules, seal and store at 4°C. (May be stored in Teflon® lined screw cap vial when in use.)

7.3.5 Azeotropic Distillation of Drinking Water

Assemble the Peter's apparatus as shown in Figure 7.3. Make the 200 mL water sample 20% (w/v) in NaCl and buffer to pH 7.0 with 0.006N of phosphate ions. Heat the sample to refluxing temperature and reflux for 30 min. Transfer the 3 mL condensate from the distillate chamber to a clean glass vial (10 mL) sealable with a Teflon® lined screw cap. Rinse the still's distillate chamber with 2 mL of reagent water and also transfer this to the vial. Use a 5 mL glass syringe for transfers.

7.3.6 Calibration of Gas Bulb

The gas bulb in Figure 7.5 is calibrated by filling with reagent water and then measuring the volume of water it held with a graduate cylinder. The gas bulb volume is used to calculate the volume of external standard, perfluorotoluene, to be added to each NEWS fraction analyzed.

7.4 GC/MS/COMP ANALYSIS

Prior to beginning GC/MS/COMP analysis of NEWS compounds the analyst should consult the general procedures in Chapter 13 entitled "GC/MS/COMP Analysis Procedure - General Instructions for All Protocols."

7.4.1 Preparation of Gaseous External Standard

A 1 L gas bulb (Fig. 7.5) with a septum port and containing a Teflon coated magnetic stirring bar is flushed for 3 min (~3 L/min) with He, while the gas bulb is in a heating mantle (35°C) and positioned on a magnetic stirrer. Approximately 0.5-0.7 µL (the exact volume is recorded) of perfluorotoluene (PFT) is injected into the gas bulb and stirred for 30 min to attain complete volatilization and mixing.

7.4.2 Preparation for Analysis

7.4.2.1 GC/MS/COMP Operating Parameters--

The recommended GC/MS operating parameters are given in Table 7.2 for the analysis of NEWS compounds. Other gas chromatographic capillaries which meet the system performance criteria may be substituted for the recommended fused silica (See Section 13.4).

Table 7.2. GC/MS OPERATING CONDITIONS FOR NEWS COMPOUND ANALYSIS

GC Column	60 m DB-5 wide-bore fused silica capillary column (1.0 μ film thickness)
GC Carrier Gas	Helium
Carrier Gas Flow Rate	1.8 mL/min
Carrier Gas Sweep Velocity	28 cm/sec
Temperature Program	35°C for 5 min; 45°C for 5 min; programmed to 200°C at 4°C/min
Injector Temperature	200°C
MS Transfer Line Temperature	260°C
Injection Mode	Splitless
Injection Volume (PFT vapor in helium)	1.0 mL
Ionizing Energy	70 eV
Ion Source Temperature	250°C
Scan Range	35-400
Scan Speed	Scan 0.95 sec, hold 0.05 sec

7.4.2.2 MS Calibration--

The mass spectrometer is calibrated using the manufacturer's recommended approach (Chapter 13). The acceptability of the calibration results is verified upon analysis of the system performance standard (SPS).

7.4.2.3 Analysis of SPS (Quality Control)--

Prior to analysis of the SPS or any samples, the sorbent trap (Tenax GC) on the purge and trap system is conditioned at 220°C for 30 min initially each day when the purge and trap system is to be used. The valve (E, Fig. 7.1) is in position "A." The temperatures on the system are set as indicated in Table 7.3.

The system performance mixture (Table 7.1) is analyzed initially each day before any samples are run. A 0.2 μL injection is made directly into

Table 7.3. TEMPERATURE SET-POINTS ON PURGE AND TRAP SYSTEM (FIG. 7.1)

Parameter	Temperature (°C)
• 6-port valve (8)[a]	160
• transfer line (20) from purge flask to 6-port valve	160
• transfer lines from 6-port valve to sorbent trap (21 and 22)	160
• transfer line from 6-port valve to injector block (5)	160
• GC injection port and valve (1)	200
• purge vessel temperature (outside)	95
• cryofocussing trap (2)	
- bakeout	240
- operational modes	
* cryogenic[b]	-195
* sample injection	220
• Tenax GC trap (4)	
- bakeout	220
- sample adsorption	ambient (~23)
- sample desorbtion	200

[a] ( ) denotes the item numbered in Fig. A-7 and Tables A-2 and A-3 of Appendix A.

the injection port (H, valve in position "B," Fig. 7.1) with the cryofocussing trap cooled to liquid nitrogen temperature. After 4 min the liquid nitrogen cooling of the cryofocussing trap is discontinued, the trap temperature is raised to 220°C and data acquisition is started.

The SPS raw data are extracted from the run, and test parameters calculated and plotted or tabulated (Tables 7.4 and 7.5). Up to 14 days of SPS analytical results can be historically recorded in Table 7.4. This allows the analyst to follow subtle trends which may develop, and antici-

Table 7.4. GC-MS SYSTEM PERFORMANCE TEST FOR NEWS COMPOUNDS

Dates:

Run Id Code

GC Column and Program:

Notebook References:

MS SYSTEM CHECKS

High/Low Mass Balance

Perfluorotoluene:

m/z	69	74	79	93	98	108	111	148	167	186	217	218	236	237
Abundance*	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )
Day														

Bromofluorobenzene:

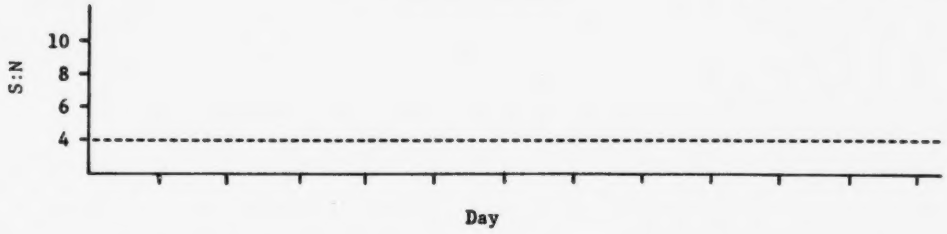
Ion	Relative Abundance Criteria	Abundance Observed Day					
50	15 to 40% of m/z 95						
75	30 to 60% of m/z 95						
95	100%						
96	5 to 9% of m/z 95						
173	<2% of m/z 174						
174	>50% of m/z 95						
175	5 to 9% of m/z 174						
176	>95%, <101% of m/z 174						
177	5 to 9% of m/z 176						

*These abundances are initially established by the MAS user and subsequently become his guideline for acceptability of calibration.

(continued)

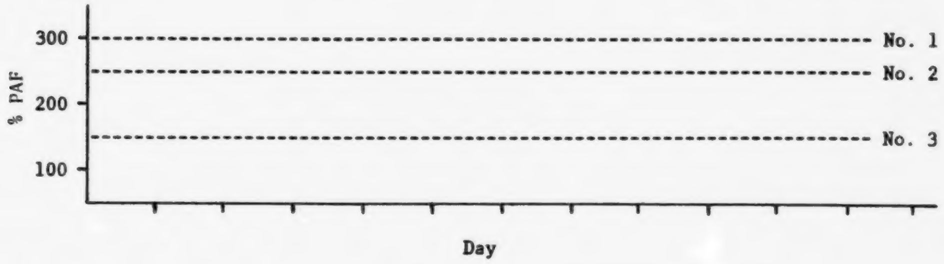
Table 7.4 (cont'd.)

Test	Components	Criteria
Limit of Detection	Trimethylbenzene	S:N (m/z 51) > 4:1

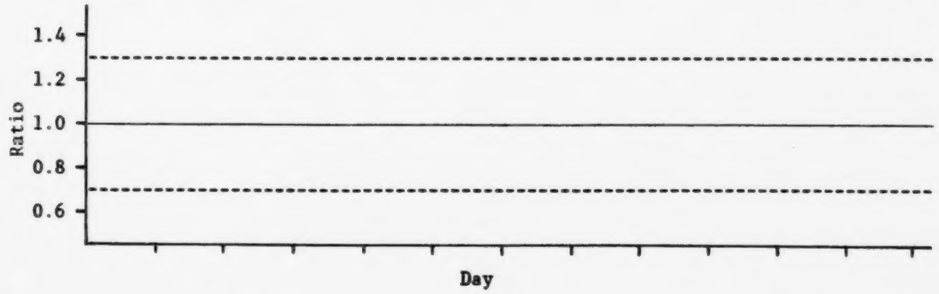


GC SYSTEM CHECKS

Peak Asymmetry	1. Acetophenone (m/z 120 or 105) ● 2. 1-Octanol (m/z 70 or 84) ▲ 3. 5-Nonanone (m/z 57) ■	% PAF < 300 % PAF < 250 % PAF < 160

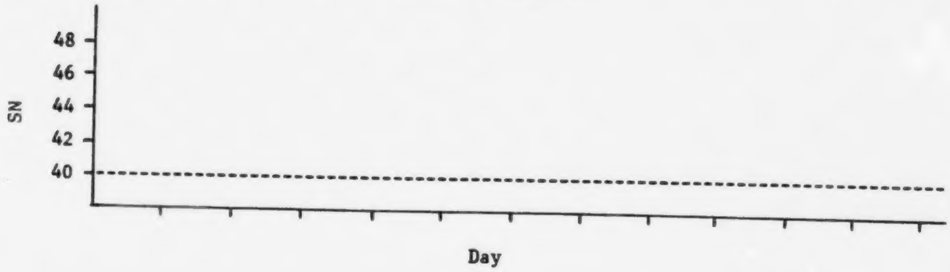
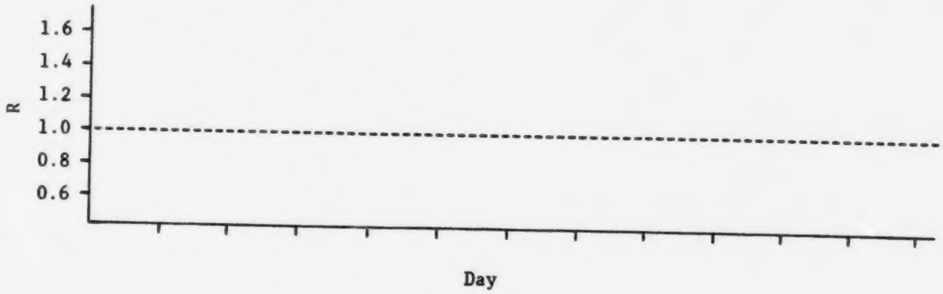
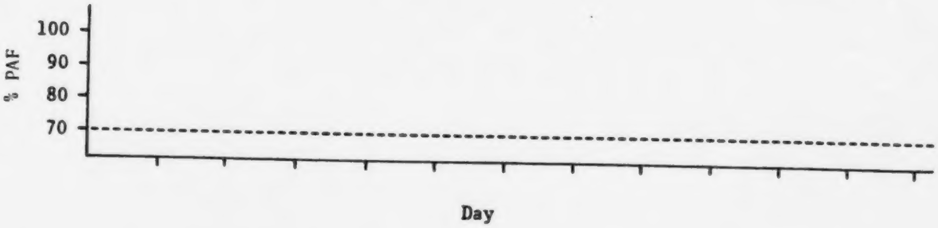


Acidity/ Basicity	1. Acetophenone (TIC) 2. 2,6-Dimethylphenol (TIC) 3. 2,6-Dimethylaniline (TIC)	 Ratio 2:1 = 0.7 to 1.3 ● Ratio 3:1 = 0.7 to 1.3 ■



(continued)

Table 7.4 (cont'd.)

Test	Components	Criteria
Separation Number	n-Octane (m/z 43) n-Decane (m/z 43)	SN ≥ 40
		
Resolution	Ethylbenzene (m/z 91) p-Xylene (m/z 91)	R ≥ 1.0
		
Capillary Capacity	n-Nonane (m/z 43)	% PAF > 70
		

COMMENTS:

Table 7.5. GC-MS SYSTEM PERFORMANCE TEST-MAS:
RMR CHECK FOR NEUTRAL WATER SOLUBLE ORGANICS (NEWS)

Date: ____________________ Run ID Code: ____________________

DATA

Standard	MW	Amount (ng)	μM	Ion (m/z)	Area (Run 1)	Area (Run 2)
Perfluorotoluene (PFT)	236			186		
				236		
d_9-t-butanol	83			64		
d_5-nitrobenzene	128			82		
				128		

MATRIX OF STANDARD ION RMRs

Standard	Ion	186	236	64	82	128
PFT	186	-				
	236		-			
d_9-t-butanol	64			-		
d_5-nitrobenzene	82				-	
	128					-

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

pate any GC or MS maintenance required. Calculation of test parameters and discussion of their use is given in Chapter 13.

A check of RMRs on a daily basis is also calculated from SPS raw data and tabulated (Table 7.5) and compared to that set developed for the historical data bank. The usage of this data is also discussed in Chapter 13.

7.4.3 Determination of RMRs

RMRs for the user's data bank (as opposed to the daily RMR check discussed in the above paragraph) are determined by injecting 1 µL of the standard solution containing NEWS compounds and deuterated standards (see Section 7.3.4) into a 10 mL empty purge vessel (Fig. 7.2) and performing a "dry" purge.

A clean 10 mL purge flask is assembled in place on the purge and trap stand (Fig. 7.1). The line at valve C is disconnected. Valves A and B are closed, and C and D are open. Purge the vessel for 5 min with He at 20 mL/min. Stop purge and connect line to valve C. Inject 1 µL of the standard solution through valve A into the purge vessel's air space. Close valve A. Purge vessel for 15 min at 20 mL/min. After 7 min from commencement of purging, introduce the external standard into the cryofocussing trap as described in Section 7.4.4.3 below and continue from that point with remainder of the purging, traping and analysis steps. The GC/MS/COMP operating parameters were given in Table 7.2. Table 13.8 may be used to tabulate RMR raw data.

7.4.4 Analysis of Blanks, Controls, Samples, and Surrogates

7.4.4.1 Drinking Water--

Add 1 g NaCl (free of NEWS compounds) to a clean 10 mL purge vessel (Fig. 7.2) and assemble on the purge and trap stand (Fig. 7.1). With line at C disconnected and valve B closed, prepurge with purified helium for 2 min at 20 mL/min and then reconnect. Valves B and D should then be closed, valves A and C open. Transfer the 5 mL condensate obtained from the Peter's still (Section 7.3.5) to the purge vessel by using a 10 mL glass syringe and injecting through valve A on the purge vessel. Close valves A and C and shake the purge vessel until the NaCl has dissolved. Proceed to Section 7.4.4.3 for elevated temperature purge, trap, and GC/MS/COMP analysis.

7.4.4.2 Other Water Types--

Add 2 g NaCl (free of NEWS compounds) to a clean 10 mL purge vessel (Fig. 7.2) and assemble on the purge and trap stand (Fig. 7.1). With line at C disconnected and valve B closed, prepurge the salt for 2 min at 20 mL/min. Do not reconnect line at C. Transfer a 10 mL water sample to the purge vessel by injecting through valve A. Close valves A, C, and D and shake the purge vessel in place until the NaCl has dissolved. Open valve C and D and prepurge the water sample with helium for 3 min at

20 mL/min to remove VO compounds. Reconnect line at C. Proceed to Section 7.4.4.3.

7.4.4.3 Elevated Temperature Purge, Trap, and GC/MS/COMP Analysis--

Wrap the tubular heater (I, Fig. 7.1) with the thermocouple around the purge vessel and heat the water to 95°C (the actual water temperature is 80°C, since the thermocouple is next to the heater) for 5 min. [Be sure that the Tenax GC trap is at ambient temperature and the 6-port (E) and the purge gas (F) valves are in position "A."] The water sample is then purged with helium at 30 mL/min for 20 min with valves C and D open and A and B closed. Shut off the purge vessel temperature controller. When purging is complete, an 8 min "dry" purge (30 mL/min) of the Tenax GC trap is performed with the 6-port valve (E) in position "A" and the purge gas valve (F) in position "B." All valves except D on the purge vessel are closed during the "dry" purge step.

A 1.0 mL gas sample of PFT is injected into the injection port (G, injection valve in "B" position, Fig. 7.1) to introduce the external standard into the cryofocussing trap (which has been cooled to liquid nitrogen temperature, a process which may take 5 min) beginning 5 min after "dry" purging has commenced.

Several points need to be emphasized for obtaining reproducibility of PFT GC/MS response:

(1) The needle on the gas sampling syringe (10 mL) should have a rounded solid tip with a side port for gas entrance and exit. (The large gauge of a regular tapered needle point cuts slices out of septa.)

(2) The septum on the GC must be tight to remain leak-free. A decrease in column head-pressure can be observed on the gauge during injection if the septum is not very tight, even when the side-port needle is used.

(3) Any septum-sweep flow in the injection port must be closed off. As much as 30% of the 1 mL injected can be lost out the septum purge vent.

(4) The Teflon plunger and/or surfaces in the syringe can retain about 10% of the previous PFT analyte. This is, if 1 mL PFT vapor gives an area of 100,000 counts, a repeat 1 mL injection

of air will yield about 10,000 counts of PFT. If the syringe plunger is pulled up to 2 mL and depressed to 0 mL 5-6 times the area count on a subsequent 1 mL injection of air is less than 2,000 counts.

(5) The syringe should have a closure such that the 1 mL PFT vapor sample can be locked in the syringe and the sample pressurized before injection into the GC; i.e., the plunger at 1 mL graduation is depressed to 0.7 mL before the needle is injected into the injection port to equalize pressure in the syringe (follow manufacturer's instructions).

By observing these precautions, the precision of PFT GC/MS response can be maintained at less than 10% relative standard deviation throughout an 8 hr period. However, the PFT gas should be produced fresh each day.

At the end of the "dry" purge step the 6-port valve (E) is rotated to the "B" position (desorb mode), and the injection port (G) valve to the "A" position (Fig. 7.1). The Tenax GC trap is desorbed by heating to 200°C with the He flow at 15 mL/min. After 8 min, the liquid nitrogen cooling of the cryofocussing trap is terminated, the injection port valve is rotated to the "B" position, the temperature on the cryofocussing trap is raised to 220°C, and data acquisition started with the GC program conditions given in Table 7.2.

The purge vessel is removed and thoroughly rinsed with deionized water, using suction to pull water through the frit, to remove residual salt before caking ensues.

7.4.4 Sample Data Analysis

Qualitative and quantitative procedures are described in Chapter 13. Table 7.6 may be used to tabulate sample analyte raw data prior to using the MASQUANT software program or manual quantitation.

Table 7.6. RAW ANALYTE DATA FOR NEWS FRACTION

Date: ____________ Run I.D. Code: ____________

Sample Identification: ________ Notebook Reference: ________

Vol. Water (L): ________ Vol. Water Processed (mL): ____
(to which iS were added)

STANDARDS

	I.D. No.	Spectrum No.	Weight* (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
Perfluorotoluene				186,236:	,		,
d_9-t-Butanol				64:	,		,
d_5-Nitrobenzene				82,128:	,		,

*Amount of internal standard in volume of water processed

ANALYTES Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Areas	MW_x^a
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{A_y} \times \frac{ng_y}{RMR_{x/y} \cdot MW_y}$$

$$K = \frac{ng_y}{RMR_{x/y} \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

CHAPTER 8

BATCH LIQUID-LIQUID EXTRACTION AND ANALYSIS OF SEMIVOLATILE STRONG ACIDS (ESSA)

8.1 INTRODUCTION

8.1.1 Principle of the Method

Batch liquid-liquid extraction (BLLE) is used to extract and concentrate semivolatile carboxylic acids and some of the more acidic phenolic compounds from aqueous samples. The sample is collected in a 1 L bottle with 20 mL of methylene chloride. The extraction is performed by adjusting the pH to 10 and extracting in a separatory funnel with 100 mL of methylene chloride. This extract contains neutral and basic compounds and is discarded. The pH of the sample is then adjusted to <4 with HCl and the sample is made 0.1 M in HCl. Sodium chloride is added to give a final concentration of 20% w/v. The sample is then extracted two times with 300 mL of methyl-t-butyl ether (MBE). The ether extracts are combined, dried by passing through a sodium sulfate column, and evaporated to ~4 mL using a macro Kuderna-Danish evaporator. The sample is further concentrated to 1 mL by nitrogen blowdown using a modified micro-Snyder column. A 0.3 mL aliquot of gaseous HCl in methanol (1 M) is added to facilitate derivatization of halogenated acids. The acids are converted to their methyl esters or ethers using diazomethane reagent in diethyl ether. After completion of the reaction the solvent volume is reduced to 1.0 mL for all waters except surface and drinking water which are evaporated to 0.5 mL. This extract is submitted for GC/MS analysis.

8.1.2 Detection Limit and Sample Size

Since all water types have the same initial ESSA sample size, detection limits will depend upon the final volume of sample concentrate. If the detection limit for GC/MS analysis of each extractable organic is estimated at 10 ng and samples are concentrated from an original volume of 1 L, then using a 1 μL injection size, nominal detection limits for specific water types are as follows:

5 ppb in surface and drinking waters (0.5 mL final volume)

10 ppb in industrial and municipal wastewaters and energy effluents (1.0 mL final volume)

If the sample cannot be concentrated to the specified volume due to high levels of contaminants, nominal detection limits are proportionately higher. Usually the limits of detection for quantitative analysis of target compounds are a factor of five lower.

8.1.3 Interferences

The pre-extraction of the sample with methylene chloride at pH 10 reduces major interferences from neutral and basic organic compounds.

8.1.4 Recoveries and Scope

Table 1.6 in Chapter 1 presents average percent recovery and standard deviations for a variety of ESSA compounds.

8.2 APPARATUS AND REAGENTS

The following materials are required for processing a set of nine samples plus two procedural blanks. Nine is the maximum number of field samples which should be processed at a time. The samples which should be run during these analyses are listed in Table 4.1. The three procedural blanks should be run before any samples are collected or processed. The extraction/ concentration procedure will require two working days for a set of eight samples and one procedural blank.

(1) Nine separatory funnels (2000 mL) with Teflon stopcocks.

(2) Nine 1 L glass bottles with Teflon lined screw caps for collecting the extracting solvent prior to solvent evaporation.

(3) One 1 L graduated cylinder for measuring sample volumes.

(4) Nine chromatographic columns for sodium sulfate solvent drying. 300 mm × 10 mm i.d. with a glass wool plug at the bottom. Approximate dimensions are shown in Figure 8.1.

(5) One Soxhlet extractor (50 mL capacity minimum) for extracting glass wool.

(6) Eighteen glass funnels to fit chromatography columns and separatory funnels.

(7) Pre-extracted glass wool (~50 mL) for drying columns. Glass wool should be precleaned by extracting in a Soxhlet overnight with methylene chloride.

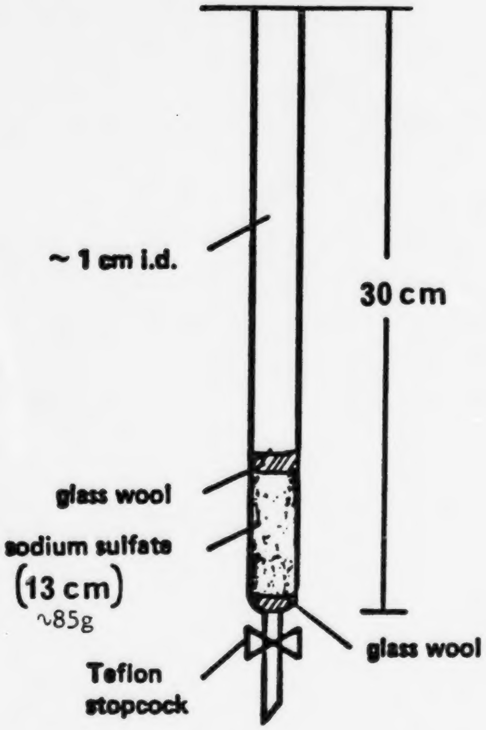


Figure 8.1. Sodium sulfate drying column.

(8) Boiling chips - Hengar granules are ground with a mortar and pestle and sieved to obtain a 60/80 mesh. These granules are cleaned by extracting ∿5 g of chips three times with ∿150 mL methylene chloride.

(9) Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

(10) Balance - analytical, capable of accurately weighing to the nearest 0.001 g.

(11) One manifold with a temperature controlled water bath for nitrogen blowdown. A manifold with nine spaces is preferred but not essential.

(12) Pasteur pipettes.

(13) One 5 mL pipette for spiking water samples with internal standard solution.

(14) Eight glass vials (1 dram) with 13 mm screw caps (Supelco 2-3213) and Teflon lined rubber septa (Supelco 2-3216).

(15) Diazomethane generation/distillation apparatus (Aldrich Macro Diazoid set Z10,851-0, Aldrichimica Acta 14 No. 3 1981) all clear glass seals (Caution the presence of ground glass or scratched glass may catalyze explosive reaction of diazomethane).

(16) Four ring stands or ring supports suitable for supporting a 2000 mL separatory funnel.

(17) Nine 10-15 mL centrifuge tubes with screw caps.

(18) One gas chromatographic column - 30 m x 0.32 mm I.D. DB-5 (1.0 μ film thickness) fused silica capillary column.

(19) Ten mL volumetric flasks for preparing standard solutions.

(20) Syringes for preparing standard solutions -

(21) Graduated pipettes for preparing standard solutions.

(22) Thirty 15 mL glass vials with Teflon lined screw caps for storing standard solutions.

(23) One gas chromatograph suitable for capillary column chromatography with flame ionization detection and all required accessories including syringes, gases, and a strip chart recorder.

(24) Nine Kuderna-Danish (K-D) apparatus. The apparatus consists of:

    (a) Three-ball macro-Snyder columns (Kontes No. K503000) - 300 mm length with 24/40 joints.

    (b) Evaporation flask (Kontes No. K570002) - 500 mL with 24/40 top joint and 19/22 lower joint.

    (c) Concentrator tube (Kontes No. K570000) - 4 mL graduated, with 19/22 joint. Attach to evaporator flask with springs (Kontes No. K503000-0232). Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

(25) Materials and Reagents

    (a) 1.5 L methylene chloride (Burdick and Jackson, distilled in glass).

    (b) 80 mL concentrated HCl (high purity).

    (c) 100 mL 6N NaOH. Weigh 24 g of NaOH into a 100 mL volumetric flask and dilute to the mark with reagent water. (High purity).

(d) 80 g anhydrous sodium sulfate.

(e) 5 mL internal standard solution (Table 4.2, Chapter 4 or if available, NBS Ampoules as given in Table 4.6, Chapter 4).

(f) 1 mL external standard solution: 4-fluoro-2-iodotoluene (30 mg) and 2-fluorobiphenyl (30 mg) in 10 mL CH_2Cl_2.

(g) 1 mL surrogate standard solution (Table 4.4, Chapter 4).

(h) 1 mL system performance solution (Table 8.1).

(i) 5 L methyl t-butyl ether (MBE) (Burdick and Jackson, distilled in glass).

(j) sodium chloride, 1.6 kg.

Table 8.1. GC/MS SYSTEM PERFORMANCE SOLUTION FOR ESSA[a]

Compound	Density (@ 20°C)	Concentration µg/mL in Methanol
2,6-dimethylphenol	S[b]	300
acetophenone	1.030	310
1-tetradecanol	0.823	250
1-octadecene	0.789	240
n-octadecane	0.777	230
DFTPP	S	500
n-eicosane	S	300
n-heneicosane	S	300
methyl stearate	S	10
d_{13}-heptanoic acid (methyl ester)	0.948	280
d_5-benzoic acid (methyl ester)	1.13	340
4-fluoro-2-iodotoluene	0.883	260
methyl decanoate	0.873	870

[a] See Table 8.6 for the function of each test component in the system performance evaluation.

[b] Solid at room temperature.

(k) 5 mL gaseous HCl in methanol (1.0 M)

(l) p-tolysulfonylmethylnitrosamide (Diazald®, Aldrich).

(m) 10 mL 60% KOH (w/v). Dissolve 6 g KOH in 10 mL reagent water.

(n) Diethyl ether (peroxide free). To insure peroxide free ethyl ether dry pack 25 g of Woelm W200 Basic Alumina Super I into a glass column 100 cm × 20 mm i.d. Eulte with 500 mL of ethyl ether discarding the first 25 mL and collecting the remaining ether in an amber glass bottle. Add absolute ethanol (2% v/v) to preserve the solvent and cap with a Teflon lined screw cap. Store cold (4°C).

(o) Reagent water - reagent water is defined as water that does not produce a background interference at the limit of detection. A commercial water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

(p) 5 mL phenolphthalein solution (0.05 g/50 mL ethanol + 50 mL water).

(q) 1% formic acid solution in diethyl ether.

(r) Test external standard - 2.0 mg/mL of benzyl acetate in MBE.

8.3 PREPARATION FOR ANALYSIS

8.3.1 Preparation of System Performance Standard (Table 8.1)

(1) Prepare individual stock solutions (12 mg/mL).

- solids - accurately weigh 0.120 g of pure material, dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.
- liquids - with a 250 µL syringe accurately measure 120 µL of pure liquid; dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.

(2) Prepare a secondary standard. With a 1 mL graduated pipette accurately measure 0.25 mL of each stock standard into a 10 mL volumetric flask. For DFTPP measure 0.42 mL. Measure 0.83 mL of methyl decanoate. Dilute to volume using methylene chloride.

This should give the concentrations shown in Table 8.1. Transfer into a Teflon sealed screw cap bottle. Store at 4°C.

(3) If compound purity is 96% or greater, the weight can be used without correction to calculate the concentration of stock standards. If the compound is less than 96% pure, it cannot be used as a standard. Transfer the stock standards into Teflon sealed screw cap bottles, store at 4°C.

8.3.2 Cleaning of Materials

(1) All glassware to be used should be washed with Amway S-A-8 laundry compound (or equivalent), rinsed with deionized water and heated for a minimum of 4 hours at 500 to 550°C. All cleaned glassware should be capped immediately or covered with foil to prevent contamination.

(2) Teflon liners and Teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. The sonicated liners are vacuum-oven (~20 inches of water) dried for 3 to 5 hours at 70° and stored in clean, Teflon lined screw cap bottles.

(3) Anhydrous sodium sulfate is cleaned and dried prior to use. Approximately 1000 g of material is needed to process nine samples and is the minimum amount of material which should be prepared. The following instructions describe procedures based on 1000 g of material; however, it is possible to prepare larger batches and store the prepared material in a sealed Erlenmeyer flask. If a larger quantity of material is prepared, volumes for rinsing should be adjusted accordingly.

Sodium sulfate (1000 g) is placed in a 1 L Erlenmeyer flask with a ground glass joint. Methylene chloride (500 mL) is added, the flask is swirled for 5 minutes, and the solvent decanted. The procedure is repeated two additional times. To dry the sodium sulfate, the cleaned material is heated in the flask in a drying oven at 130°C overnight. The clean, dried material can be stored in the drying oven, or stoppered and stored in a dessicator.

To prepare drying columns, a small piece of pre-extracted glass wool is placed in the bottom of the chromatography column, 13 cm of sodium sulfate is poured into the column, followed by an additional plug of pre-extracted glass wool. Columns should be used immediately or stored in a drying oven at 130°C. Rinse the Na_2SO_4 with 50 mL of extracting solvent just prior to using the column.

(4) Glass wool is cleaned by placing in the extraction chamber of a Soxhlet extractor and extracting overnight with methylene chloride. The glass wool is placed in a wide mouth jar and the solvent removed either under nitrogen stream or by warming. Cap and store until needed.

8.3.3 GC/FID Performance Evaluation

Prior to analyzing procedural blanks, acceptable performance for the GC/FID system must be demonstrated.

(1) Analyze the GC/MS system performance solution (SPS) as specified in Table 8.2. Figure 8.2 shows a total ion chromatogram of this mixture analyzed by GC/MS under similar conditions which may be used to identify test components in the standard.

Table 8.2. GC/FID OPERATING CONDITIONS FOR EXTRACTABLE SEMIVOLATILE STRONG ACIDS (ESSA)

GC Column	30 m DB-5 (1.0 μ film thickness) fused silica, wide bore (0.34 mm I.D.), capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min column; 15:1 split
Temperature Program	50°C/5 min to 250°C @ 4°/min
Injector Temperature	250°C
Detector Temperature	260°C
Injection Volume	1.0 μL

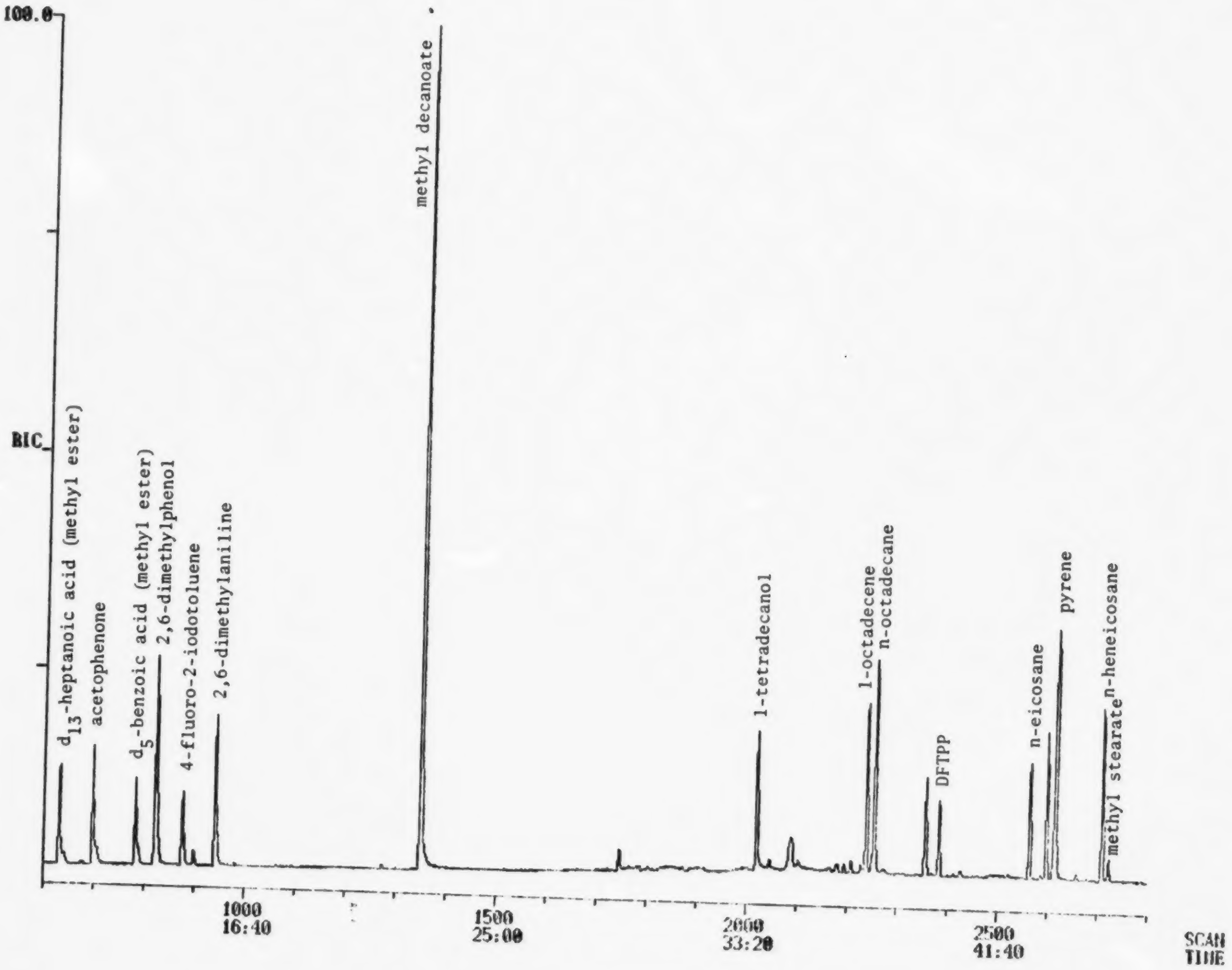


Figure 8.2. Total ion chromatogram of ESSA SPS.

(2) Measure peak asymmetry or tailing for acetophenone of 1-tetradecanol using the percent peak asymmetry factor (PAF):

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

where

B = the width of the back of a chromatographic peak to the perpendicular from the peak measured at 10% above baseline.

F = the width of the front of a chromatographic peak to the perpendicular from the peak measured at 10% above baseline.

PAF should measure less than 200% for both acetophenone and 1-tetradecanol.

(3) Check the acidity/basicity of the column by the peak area ratios determined by integrator or triangulation of 2,6-dimethylphenol to acetophenone. A ratio of 0.7 to 1.3 is acceptable.

(4) Sensitivity is checked by measuring signal to noise ratios for meatyl stearate. A signal-to-noise ratio of 10 is acceptable.

8.3.4 Procedural Blanks

For each lot of materials and reagents that are used, a set of three procedural blanks are required. Table 8.3 identifies these blanks and defines their purpose. These blanks must be processed and analyzed by GC/FID prior to processing any samples. If a large number of samples are to be processed, it is advantageous to prepare large lots of materials and reagents thereby reducing the number of blanks which must be run. Procedural blank 1 must also be run every time a batch of samples is processed and analyzed.

(1) Prepare a separatory funnel rinse with 100 mL methylene chloride and discard. Perform extraction with reagent water described in step 8.4.1.5 through 8.4.1.20 and the derivatization procedure in section 8.4.2.

(2) Procedural blank 2 is prepared from reagent water (920 mL) placed in a solvent bottle and extracted as a sample.

(3) Procedural blank 3 is prepared in the same way as 2 except that the reagent water is spiked with 1 mL each of the sulfuric acid,

Table 8.3. PROCEDURAL BLANKS

Blank	Description
Procedural Blank 1	detects contamination in solvents and glassware
Procedural Blank 2	detects contamination in reagent water
Procedural Blank 3	detects contamination in sulfuric acid, sodium hydroxide, and sodium thiosulfate solutions used during sample collection and analysis

sodium hydroxide, and thiosulfate solution to be used in sample collection. This blank is also extraction as a sample.

(4) Add 10 µL of the GC external standard to each blank concentrate after derivatization and concentration to 1 mL. Agitate the K-D receiver to give a uniform distribution of standard in the concentrates.

(5) Analyze the samples by GC/FID using the conditions in Table 8.2. If significant contamination (>20% relative to external standard) is present in procedural blank 1, reclean glassware and check solvents and sodium sulfate. If contamination is present in procedural blank 2 which is not in procedural blank 1 use another source of reagent water. Procedural blank 3 contamination should measure less than 20% relative to the external standard. Excessive contamination of this blank requires procurement of fresh solutions of sulfuric acid, sodium hydroxide, and sodium thiosulfate. If the contamination persists, obtain materials from another lot/source.

8.3.5 Preparation of Gaseous HCl:Methanol

HCl:methanol solution is usually prepared in large batches and may be stored in the refrigerator up to 2 weeks between use. Because HCl is corrosive, the entire operation should be performed in a well-ventilated hood. Methanol (500 mL) is added to a 1 L amber glass bottle, and the bottle is chilled in an ice bath. Gaseous HCl is bubbled through the methanol as illustrated in Figure 8.3. HCl is allowed to dissolve into

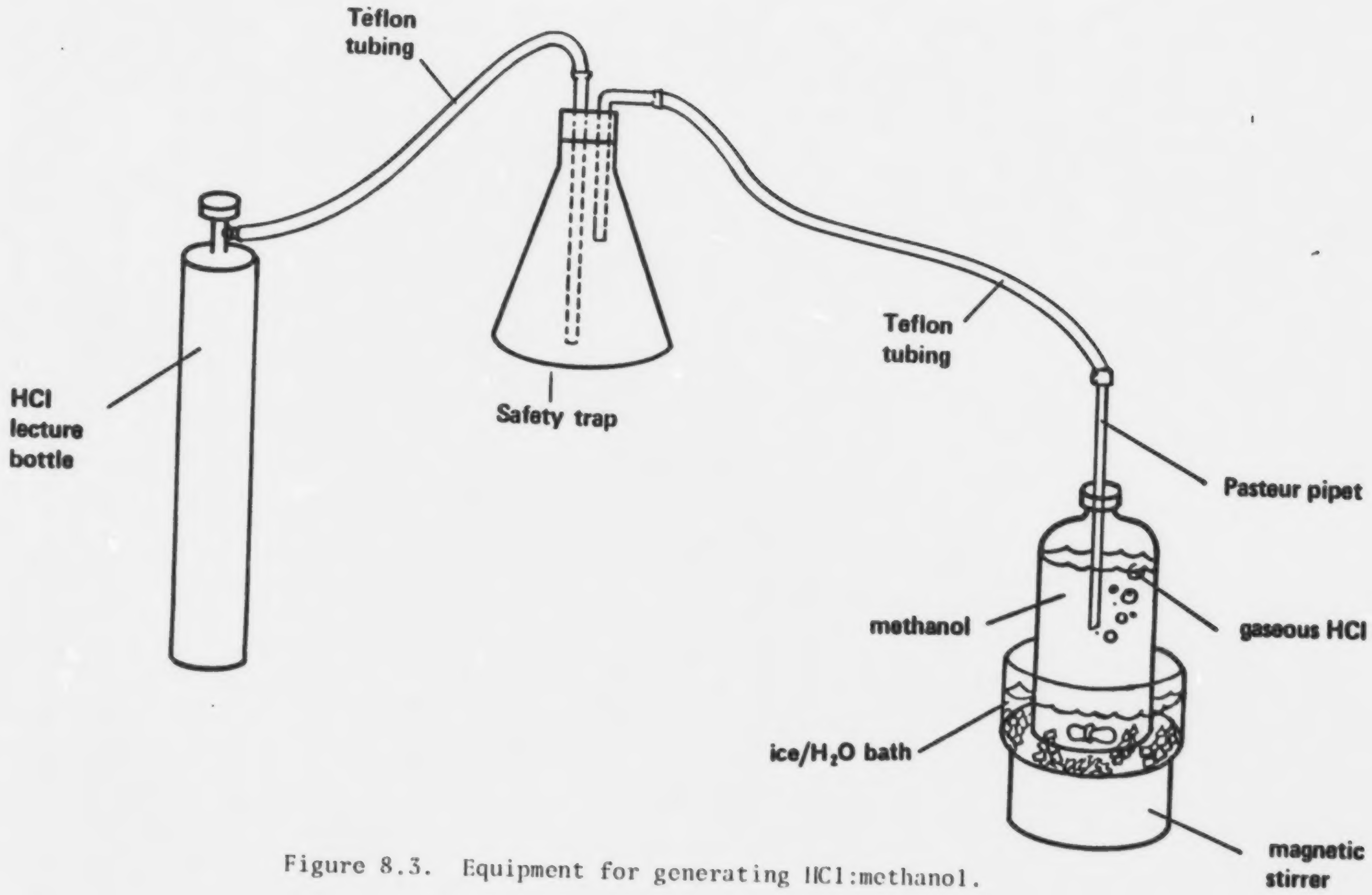

Teflon tubing
HCl lecture bottle
Safety trap
Teflon tubing
Pasteur pipet
methanol
gaseous HCl
ice/H_2O bath
magnetic stirrer

Figure 8.3. Equipment for generating HCl:methanol.

the methanol for ~15 minutes. The HCl concentration of the methanol solution is determined by adding 1 mL of the methanol solution to 20 mL of distilled-deionized water, and titrating to the phenolphthalein endpoint. A normality from 0.8 to 1.2 is acceptable for the analytical operation. If the solution is stored, the normality should be checked before use. Smaller amounts may be prepared in a similar manner.

8.3.6 Quality Control on the Derivatization Reaction

Before beginning the analysis of any samples, a procedural blank, a derivatization control, and a derivatization blank are processed through the procedure. The procedural blank is reagent water spiked with internal standards processed through the procedure in 8.4. The derivatization control is a 1.0 mL aliquot of a standard solution, (Table 8.4) with 0.30 mL of gaseous HCl (1.0 M)/methanol added. The derivatization blank is 1.0 mL of MBE with gaseous HCl(1.0 M)/methanol added. These three samples are derivatized by the procedure in 8.4.2 and analyzed by GC/FID to determine the level of the background and the conversion of the acids to methyl esters and ethers. An aliquot (25 µL) of benzyl acetate (2.0 mg/mL in MBE) is added to each sample before GC/FID analysis. The GC/FID response ratio which must be attained for the derivatization control are given in Table 8.4.

8.4 SAMPLE EXTRACTION AND DERIVATIZATION

8.4.1 Sample Extraction

(1) Mark the menicus of the aqueous liquid on the sample bottle with an indelible marker.

(2) Adjust the pH of the sample to ~10 with 6 M NaOH using narrow range pH paper to check the pH.

(3) Immediately, transfer the entire contents of the sample bottle to the 2000 mL separatory funnel.

(4) Immediately, extract (~60-120 cpm as required to avoid emulsions) for 2 minutes with 100 mL of methylene chloride and discard the methylene chloride.

(5) Immediately adjust the pH to <4 with concentrated HCl using wide range pH paper to check pH. Lapsed time from step 2 through step 5 should not exceed 15 minutes. It is advisable to work

Table 8.4. RELATIVE GC/FID RESPONSES FOR ACID SURROGATE COMPOUNDS

Compound	Densities (@ 20°C)	Concentration[a] (μg/mL)	Response Ratio[b] Range
trichloroacetic acid	S	500	0.12 - 0.15
benzoic acid	S	500	0.75 - 0.88
_n_-octanoic acid	0.906	450	0.70 - 0.87
2,4-dichlorophenol	S	500	0.56 - 0.64
2-nitro-_p_-cresol	S	500	0.78 - 0.90
2,4-D	S	500	0.52 - 0.66
2,4,5-T	S	500	0.40 - 0.65

[a] Preparation of solution -

(1) Accurately weigh or volumetrically measure with a microliter syringe each compound into a 50 mL volumetric flask (25 mg or 25 μL, respectively).

(2) Dissolve these materials in methanol and dilute to the mark.

[b] $$\text{Response Ratio} = \frac{(\text{Peak area compound})(\mu\text{g benzylacetate})}{(\text{Peak area benzylacetate})(\mu\text{g compound})}$$

The range is defined by ± 3 standard deviations of triplicate determinations. Response ratio computed using peak heights instead of areas.

with one sample at a time through these steps to minimize the time which the sample is at an elevated pH.

(6) Add 200 g of NaCl and dissolve.

(7) Add concentrated HCl to make the final concentration 0.1M in HCl. For a 1.0 L sample add 8.3 mL of concentrated HCl.

(8) Extract sample with 300 mL of methyl _t_-butyl ether.

(9) Drain aqueous layer into the sample bottle tapping the sides of the separatory funnel to release droplets which may adhere to the sides. Remove the aqueous layer as completely as possible.

(10) Drain the MBE through a glass wool plug into an amber bottle.

(11) Transfer the aqueous sample back into the separatory funnel and repeat steps 8 through 10 combining extracts.

(12) Add ~5 g of anhydrous Na_2SO_4 to the combined extracts.

(13) Rinse Na_2SO_4 drying column with 25 mL of MBE.

(14) Transfer ~1/2 the extract through the Na_2SO_4 drying column into a 500 mL K-D flask equipped with 4 mL concentrator tube containing several boiling chips. The concentrator tube should be precalibrated to compensate for the volume of the boiling chips. Attach the three-ball macro-Snyder column. Place the K-D apparatus on a warm water bath with the concentrator tube partially immersed in water or on a steam bath. Adjust the temperature of the water bath such that evaporating solvent causes the balls in the Snyder column to chatter actively but does not flood the chambers of the column. Under these conditions evaporation of 500 mL of MBE to 2 mL should take 20 to 30 minutes.

(15) When the liquid has reached an apparent volume of 2 mL remove the K-D apparatus from the heat and allow to cool. Remove the Snyder column.

(16) Transfer the remaining extract to the K-D apparatus through the drying column and repeat steps 14 and 15.

(17) With 1 to 2 mL of solvent, rinse the Snyder column, the evaporation flask, and its lower joint into the concentrator tube. The final volume in the concentrator tube should be approximately 4 mL.

(18) Attach a modified micro-Snyder column to the K-D concentration tube. Connect a transfer pipette by tubing to a nitrogen manifold. Place the transfer pipette in the modified Snyder column above the extract level (Figure 8.4). Gently blow a stream of nitrogen above the extract surface until the volume is reduced to approximately 1 mL. Rinse the sides of the concentrator tube with approximately 0.5 mL of solvent. Reduce volume of extract to 1.0 mL.

(19) Add 0.30 mL of gaseous HCl in methanol (1.0 M) to each extract.

(20) Fill sample bottle to the mark with water. Transfer contents to a graduated cylinder and record the volume to the nearest 5 mL.

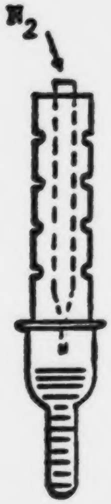


Figure 8.4. Nitrogen blowdown with modified micro-Snyder column.

8.4.2 Derivatization

Caution! Diazomethane is toxic and prone to cause development of specific sensitivity; in addition, it is potentially explosive. Hence one should wear solvent impermeable gloves and goggles while performing this experiment and should work behind a safety screen or a hood door with safety glass. Also, it is recommended that ground glass joints and sharp surfaces be avoided. Thus all glass tubes should be carefully fire-polished, connections should be made with rubber stoppers or clear glass seals, and separatory funnels should be avoided, as should etched or scratched flasks. Furthermore, at least one explosion of diazomethane has been observed at the moment crystals (sharp edges!) suddenly separated from a supersaturated solution. Stirring by means of a Teflon-coated magnetic stirrer is greatly to be preferred to swirling the reaction mixture by hand, for there has been at least one case of a chemist whose hand was injured by an explosion during the preparation of diazomethane in a hand-swirled reaction vessel.

It is imperative that diazomethane solutions not be exposed to direct sunlight or placed near a strong artificial light, because light is thought to have been responsible for some of the explosions that have been encountered with diazomethane. Particular caution should be exercised when an organic solvent boiling higher than ether is used. Because such a solvent has a lower vapor pressure than ether, the concentration of diazomethane in the vapor above the reaction mixture is greater and an explosion is more apt to occur.

Most diazomethane explosions take place during its distillation. When distilled diazomethane is required, the present procedure is particularly good because at no time is much diazomethane present in the distilling flask.

(1) Assemble the diazomethane generation/distillation apparatus (Figure 8.5).

(2) Place 10 mL fresh 60% KOH (v/v in water) in the distilling flask with 35 mL ethanol and 10 mL of diethyl ether. Add a magnetic stirring bar.

(3) Place ice/salt mixture in the condenser and place the collection flask in a ice/salt bath.

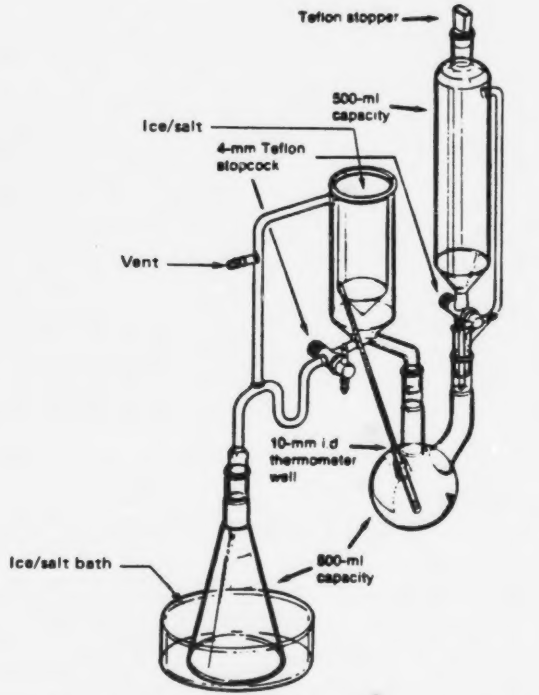

Teflon stopper
500-ml capacity
Ice/salt
4-mm Teflon stopcock
Vent
10-mm i.d thermometer well
Ice/salt bath
500-ml capacity

Figure 8.5. Z10,851-0 Macro Diazald® set (with Ŧ Clear-Seal® joints).

(4) Prepare a solution of 21.5 g (0.1 mole) of p-tolylsulfonylmethylnitrosamide (Diazald®, Aldrich) in 125 mL of diethyl ether. Larger amounts of ether may be needed to dissolve the reagent if it is especially pure or the temperature is less than 20°C. Place the Diazald® solution in the dropping funnel.

(5) The distilling flask is heated in a water bath at 70-75°C, the stirrer is started.

(6) The Diazald® solution is added at a regular rate approximately equal to the distillation rate over a 15-20 minute period. As soon as this solution has been added, add 100 mL of ether to the dropping funnel and continue to add the ether at the same rate used for the reagent.

(7) Continue distillation until the distillate is colorless.

(8) Cool the collection flask in an ice/salt bath during collection. Remove the collection flask and keep in ice bath until used. Destroy any residues of diazomethane in the apparatus by rinsing with 1% formic acid in diethyl ether.

(9) With a Pasteur pipette add the distilled diazomethane-ether solution to the sample extracts until a yellow color persists. For highly colored samples add the diazomethane solution until nitrogen evolution ceases. Additional diazomethane should be prepared as needed. Excess diazomethane is evaporated from samples using nitrogen blowdown. If excess diazomethane reagent has been prepared it should be quenched with formic acid solution (1% in ether) before discarding. The solution will be colorless when fully quenched.

(10) Concentrate the derivatized samples to 1.0 mL in the test tubes using nitrogen blowdown at ~30°C. Since excess diazomethane is still present in the samples this step must be performed in a well ventilated hood.

(11) Store the samples at 0°C until analysis.

(12) Add an aliquot (25 μL) of external standard (4-fluoro-2-iodotoluene) solution to the sample concentrate immediately before analysis.

8.5 GC/MS/COMP ANALYSIS

Prior to beginning GC/MS/COMP analysis of the extractable acid fraction the analyst should consult the general procedures described in Chapter 13 entitled "$(GC)^2$/MS/COMP Analysis-General for All Protocols".

8.5.1 Preparation for Analysis

8.5.1.1 $(GC)^2$/MS/COMP Operating Parameters

The recommended GC/MS operating parameters are given in Table 8.5 for the analysis of the extractable semivolatile strong acid fractions (ESSA). Gas chromatographic capillaries which meet the system performance criteria may be substituted for the recommended fused silica (See Section 13.4). It is recommended that this capillary be reserved for use only with sample extracts containing methyl esters and ethers of strong acids, as it tends to become acidic with continued analysis of such extracts.

Table 8.5. GC/MS OPERATING CONDITIONS FOR EXTRACTABLE STRONG ACIDS AS THEIR METHYL DERIVATIVES

GC Column	30 m DB-5 (1.0 µ film thickness) fused silica wide bore (0.34 mm I.D.) capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min - column; 9:1 split
Carrier Gas Sweep Time	85 secs (50°C)
Temperature Program	50°C/5 min; to 240°C @ 4°/min and hold
Injector Temperature	250°C
Transfer Line Temperature	255°C
Injection Mode	Splitless - 0.4 min/split
Injection Volume	1.0 µL
Ionizing Energy	70 eV
Ion Source Temperature	250°C
Scan Range	35-500

8.5.1.2 MS Calibration

The mass spectrometer is calibrated using the manufacturer's recommended approach (Chapter 13). The acceptability of the calibration results is verified upon analysis of the system performance solution (SPS).

8.5.2 Analysis of SPS (Quality Control)

Using the prescribed GC/MS conditions the SPS is analyzed and all necessary data is acquired and evaluated prior to proceeding to sample analysis. The SPS is analyzed at the beginning of each day's operation.

The SPS raw data are extracted from the run, test parameters calculated and plotted or tabulated (Tables 8.6 and 8.7). Up to 14 days of SPS analytical results can be historically recorded in Table 8.6. This allows the analyst to follow subtle trends which may develop and anticipation of GC or MS maintanence required. Calculation of test parameters and discussion of their use is given in Chapter 13.

A check of RMRs on a daily basis is also calculated from SPS raw data and tabulated (Table 8.7) and compared to that set developed for the historical data bank. The usage of this data is also discussed in Chapter 13.

8.5.3 Determination of RMRs

RMRs for the User's data bank (as opposed to the daily RMR checks discussed in the above paragraph) are determined by injecting a solution containing the derivatized internal standards and analytes. Preparation of standard solutions is the same as described in the footnote of Table 8.4.

The GC/MS/COMP operating parameters are given in Table 8.5. Table 13.8 may be used to tabulate RMR raw data.

8.5.4 Analysis of Field and Quality Control Samples

After meeting all specific criteria for GC and MS performance (Table 8.6), then analysis of standard solutions (e.g., for RMR calculations) or (ESSA) sample extracts is conducted.

A set of sample extracts may have been derived from laboratory and field blanks and controls, surrogates and collected field samples, and are generally analyzed in that respective order.

Qualitative and quantitative procedures are described in Chapter 13. Tables 8.8 may be used to tabulate sample analyte raw data prior to using the MASQUANT software program or manual quantitation.

Table 8.6. GC-MS SYSTEM PERFORMANCE TEST FOR EXTRACTABLE SEMIVOLATILE STRONG ACIDS (ESSA)

Dates: ________ ________

Run Id Code ________ ________

GC Column and Program: ____

Notebook References: ____

MS SYSTEM CHECKS

High/Low Mass Balance Ion	DFTPP Relative Abundance Criteria
51	30% to 60% of m/z 198
68	<2% of m/z 69
70	<2% of m/z 69
127	40% to 60% of m/z 198
197	<1% of m/z 198
198	100%
199	5-9% of m/z 198
275	10% to 30% of m/z 198
365	at least 1% of m/z 198
441	present, but < m/z 443
442	>40% of m/z 198
443	17% to 23% of m/z 442

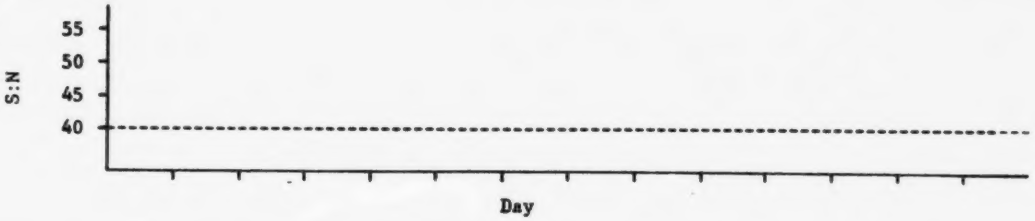
m/z	51	68	70	127	197	198	199	275	365	331	442	443	Pass/ Fail	Remedial Action
Abundance * Criteria	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )		
Day														

*These abundances are initially established by the MAS user, and subsequently become guidelines for acceptability of tune.

(continued)

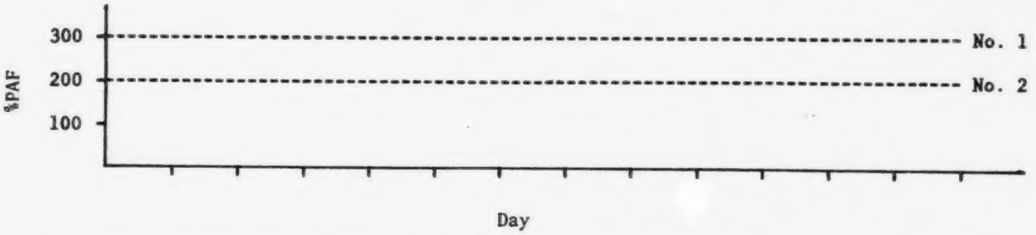
Table 8.6 (cont'd.)

Test	**Components**	**Criteria**
Limit of Detection	Methyl Stearate	S:N (m/z 74) $\geq$ 40:1

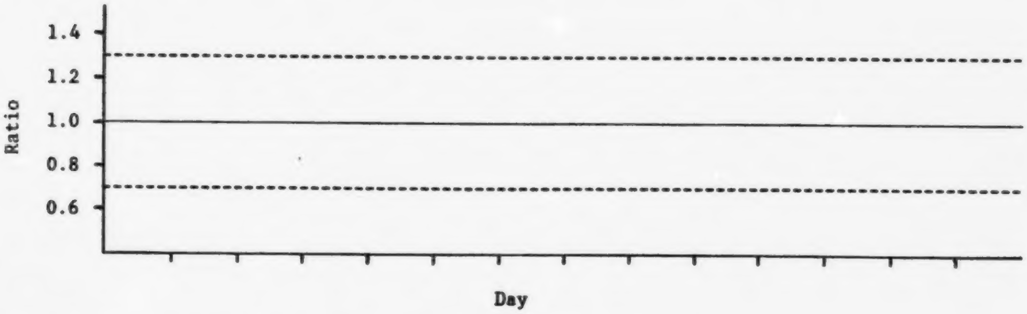


GC SYSTEM CHECKS

Test	**Components**	**Criteria**
Peak Asymmetry	1. Acetophenone (TIC) ● 2. 1-Tetradecanol (TIC ■	%PAF <300 %PAF <200



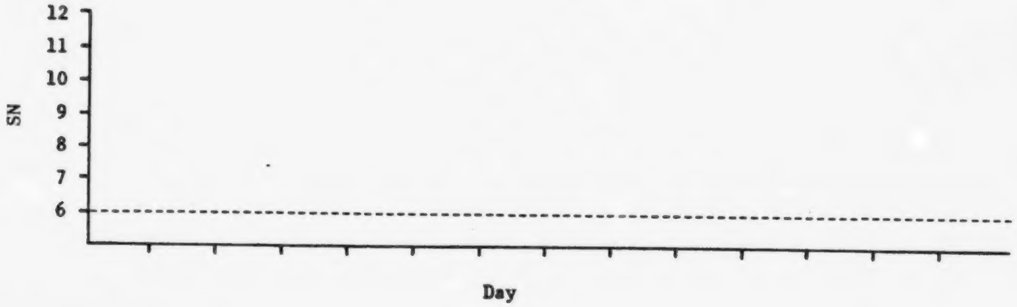
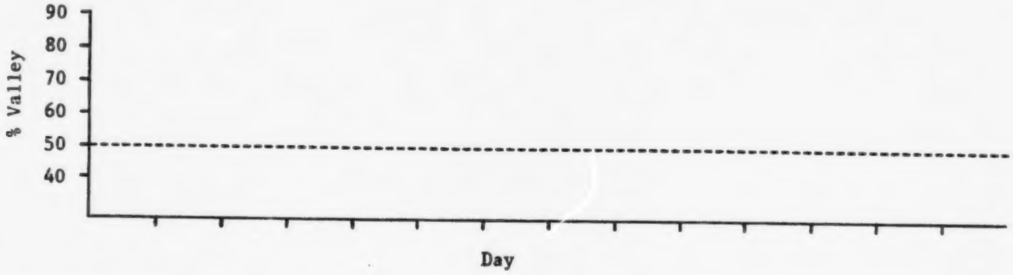
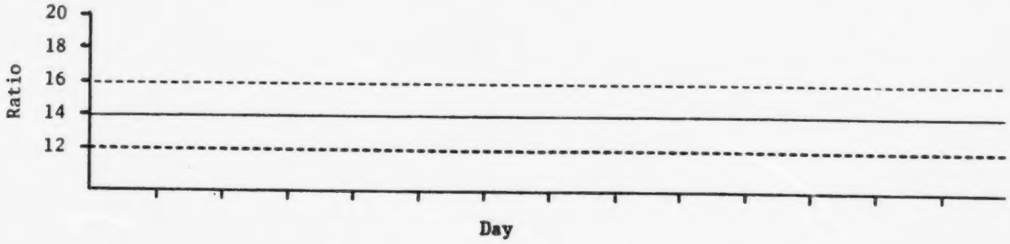
Test	**Components**	**Criteria**
Basicity*	1. Acetophenone (TIC) 2. 2,6-Dimethylphenol (TIC)	Ratio 2:1 = 0.7 to 1.3



*Note: Acidity criteria are also measured in other MAS fractions.

(continued)

Table 8.6 (cont'd.)

Test	Components	Criteria
Separation Number	n-Eicosane (m/z 43) n-Heneicosane (m/z 43)	SN ≥ 6
		
Resolution	n-Octadecane (TIC) 1-Octadecene (TIC)	R = minimum 50% Valley
		
Inertness	Methyl Stearate	Ratio m/z 74 to 298 ≤ 14:1
		

(continued)

Test	Components	Criteria
Capillary Capacity	Methyl Decanoate (TIC)	%PAF >70

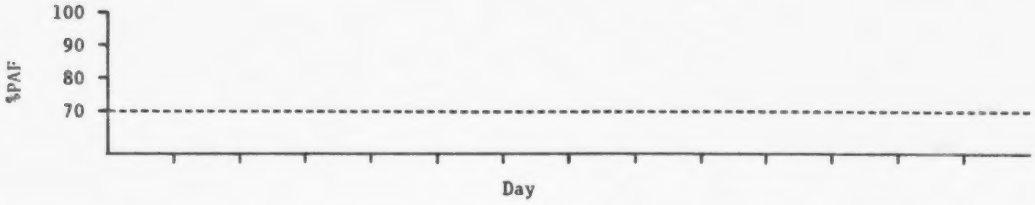


Table 8.7. GC-MS SYSTEM PERFORMANCE TEST: RMR CHECK FOR SEMIVOLATILE STRONG ACIDS (ESSA)

Date: ______________________ Run ID Code: ______________________

DATA

Standard	MW	Amount (ng)	µM	Ion (m/z)	Area (Run 1)	Area (Run 2)
4-Fluoro-2-Iodo-toluene (FIT)	236			109		
				236		
d_{13}-heptanoic acid	143			77		
				91		
d_5-benzoic acid	127			82		
				110		

MATRIX OF STANDARD ION RMRs

Standard	Ion	109	236	77	91	82	110
FIT	109	-					
	236		-				
d_{13}-heptanoic acid	77			-			
	91				-		
d_5-benzoic acid	82					-	
	110						-

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

Table 8.8. RAW ANALYTE DATA FOR ESSA FRACTION

Date: ____________ Run I.D. Code: ____________

Sample Identification: ________ Notebook Reference: ____________

Vol. Water (L): ____________ Vol. Water Processed (mL): _____
(to which is were added)

STANDARDS

	I.D. No.	Spectrum No.	Weight (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
4-Fluoro-2-Iodo-toluene	____	____	____	109,236:	______, ______	___	___, ___
d_{13}-Heptanoic Acid	____	____	____	77, 91:	______, ______	___	___, ___
d_5-Benzoic Acid	____	____	____	82,110:	______, ______	___	___, ___

ANALYTES Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Area	MW_x^a
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___
____________	____	________	___, ___:	______, ______	___

---

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{RMR_{x/y}} \times \frac{ng_y}{A_y \cdot MW_y}$$

$$K = \frac{ng_y}{A_y \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

CHAPTER 9

EXTRACTION AND ANALYSIS OF WEAK ACIDS, BASES AND NEUTRALS (WABN)

This section contains three extraction protocols (Parts A, B, and C), one of which is selected for the extraction of organics from water depending upon the water quality as assessed during sampling and sample scouting. All of the protocols allow for the recovery of weak acids, weak bases and neutral compounds (WABN) at pH 8.

The sorbent accumulator column (SC) protocol is used for extracting organics from drinking water samples. For other water samples, the emulsion index (determined just before sample processing) determines the sample's propensity for emulsification, and if that is the case, the sample is extracted using the continuous flow-under (FU) protocol. Otherwise, the batch liquid-liquid partitioning (BL) (separatory funnel) protocol is employed. A clean-up protocol (Part D) is provided for both FU and BL extracts; a screening test is prescribed in both extraction protocols to determine whether clean-up is necessary. Part E of this chapter is for GC/MS/COMP analysis of all WABN extracts.

A. SORBENT ACCUMULATOR COLUMN (WABN-SC)

9.1 INTRODUCTION

9.1.1 Principle of the Technique

This method describes the determination of pH 8-extractable (WABN) organic compounds in drinking water samples. Resin materials are used to adsorb and concentrate organic compounds from 11.5 L water samples. A nominal 0.5 ppb detection limit is achieved using this size sample. Although the method has been developed specifically for drinking water, other water types, such as some surface waters may also be processed depending upon desired sample size, organic content, and turbidity. The sample pH is adjusted to 8 and the sample passed through a 10 mL Amberlite XAD-4 macroreticular resin column. Excess water is aspirated off the column and adsorbed organics are eluted using 12 mL of methanol followed by 200 mL methylene chloride. The eluants are combined, then evaporated to a volume of 4 mL using a Kuderna-Danish evaporator and further concentrated to 0.5 mL by nitrogen blowdown using a modified micro-Snyder column. Neutral organics, weak acids, and bases are analyzed using this procedure.

9.1.2 Detection Limit

If the detection limit for GC/MS analysis is estimated at 10 ng total material for extractable organics, and samples are concentrated from an original volume of 11.5 L to a final extract volume of 0.5 mL, the overall nominal detection limit for this procedure would be 0.5 ppb for a 1 μL injection.

Detection limits may be decreased by further increasing sample size to 50 to 100 L. If this approach is taken, column accumulation should be performed in the field due to the prohibitive cost of shipping the samples. Breakthrough characteristics using large water volumes are unknown and recoveries may be significantly decreased.

9.1.3 Interferences

No interferences have been observed using the developed procedure. A procedural blank must be run prior to processing any samples to assure that contamination from resin materials, solvents, reagents, glassware, and other sources is low.

9.1.4 Precision, Accuracy, and Scope

Table 1.5 in Chapter 1 presents average recoveries and standard deviations for a variety of extractable organics. This method gives acceptable recoveries for a variety of neutral organics, as well as, weak and strong bases (*i.e.*, trimethyl aniline, tributylamine, etc.), and weak acids (*i.e.*, *t*-butyl phenol).

9.2 APPARATUS AND REAGENTS

The following materials are required for processing a set of nine samples plus two procedural blanks. Nine is the maximum number of samples which should be processed at a time. The quality control samples that should be run during these analyses are listed in Table 4.1, Chapter 4. The two procedural blanks should be run before any samples are collected or processed. The extraction/concentration procedure will require two working days to process nine samples.

(1) Nine glass accumulator columns ~1 cm I.D. × 20 cm with a teflon stopcock and a 24/40 ground glass joint at the top. Each chromatography column should have an adaptor (Figure 9.1) for siphoning the samples onto the resin.

(2) One vacuum aspirator to start sample flow through the resin column.

(3) Nine separatory funnels (250 or 500 mL) with teflon stopcocks and ground glass joints (24/40) to connect to the resin column (Figure 9.2).

(4) One 10 mL graduated pipette with the tip cut off for measuring and transferring resin material.

(5) Nine 500 mL amber bottles with Teflon lined screw caps.

(6) Glass wool (~50 mL) for resin. Glass wool should be precleaned by extracting in a Soxhlet overnight with methylene chloride.

(7) Boiling chips - Hengar granules are ground with a mortar and pestle and sieved to obtain a 60/80 mesh. These granules are cleaned by extracting ~5 g with 150 mL of methylene chloride.

(8) One Soxhlet extractor for cleaning resin material and glass wool. A minimum 200 mL capacity is required. However, resins can be processed in large batches and stored; therefore a larger size preferred.

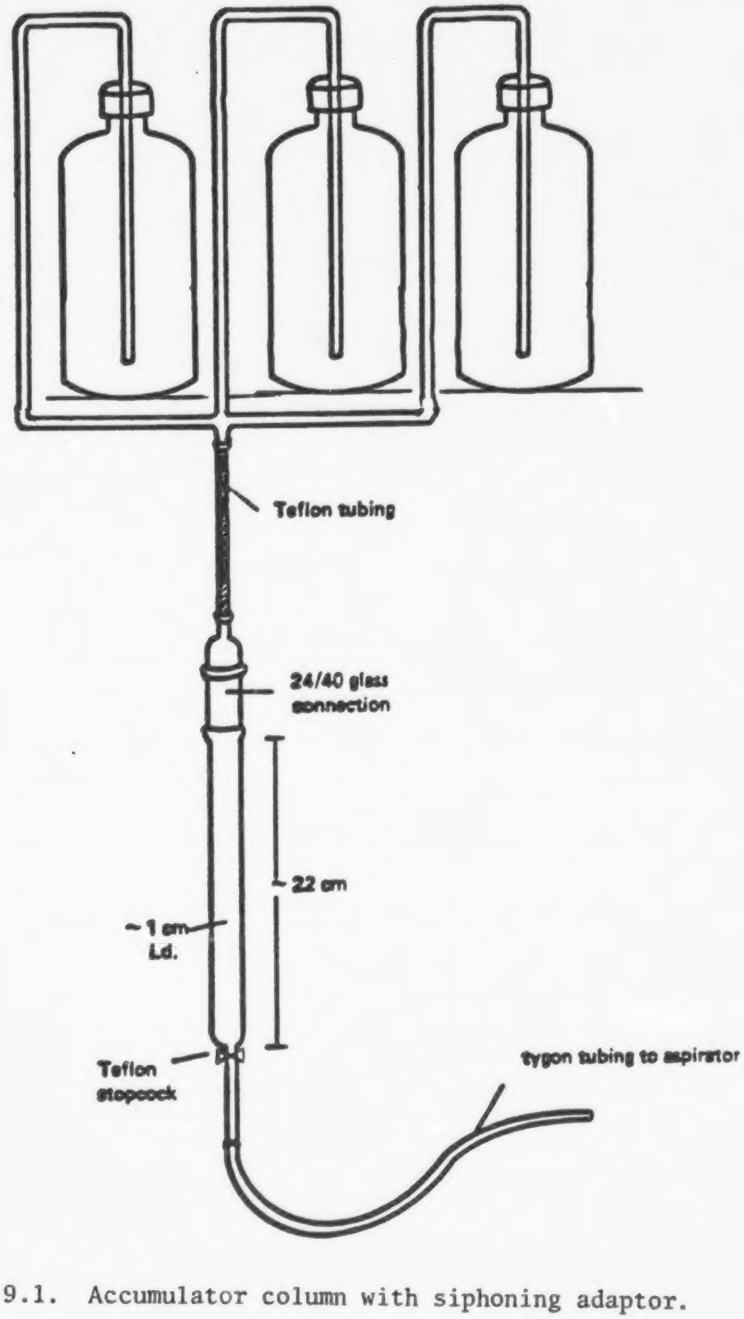

Teflon tubing
24/40 glass connection
~22 cm
~1 cm i.d.
Teflon stopcock
tygon tubing to aspirator

Figure 9.1. Accumulator column with siphoning adaptor.

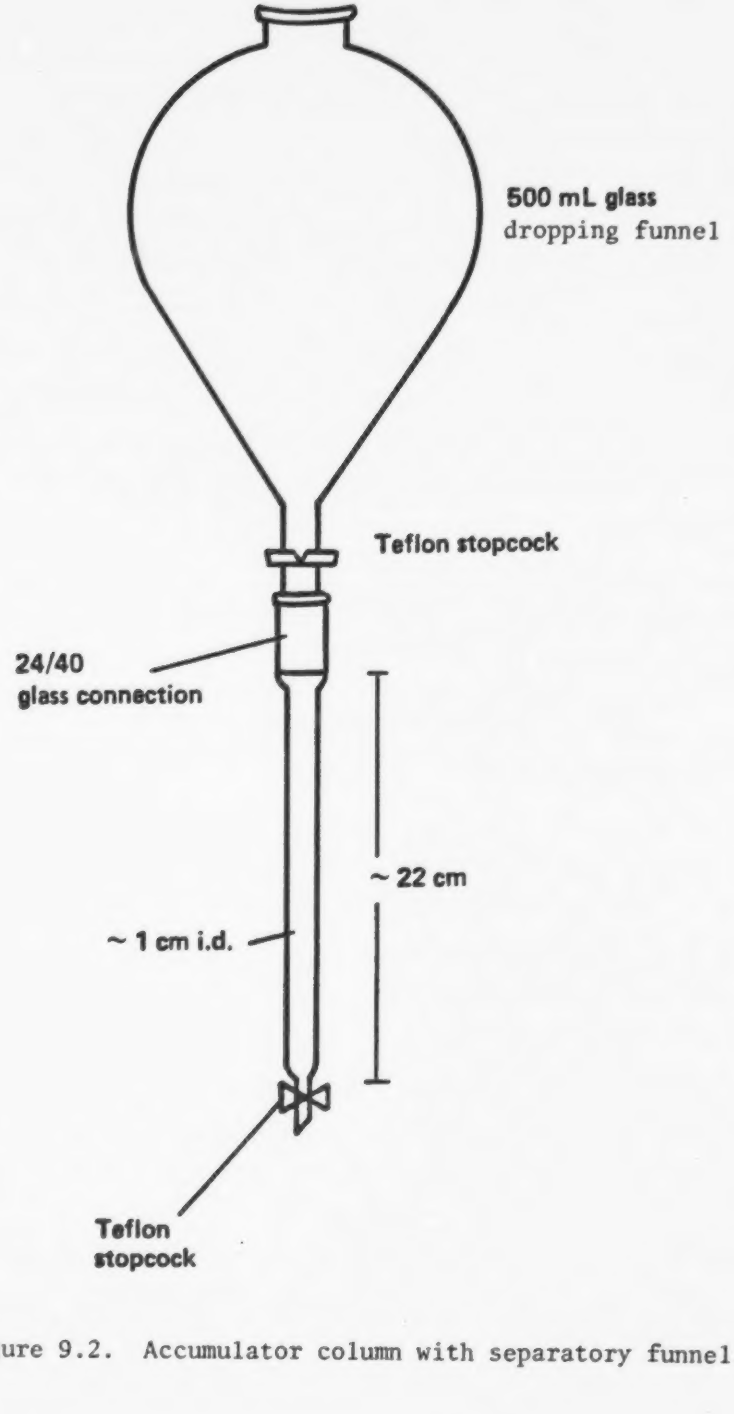

500 mL glass
dropping funnel
Teflon stopcock
24/40
glass connection
~ 22 cm
~ 1 cm i.d.
Teflon
stopcock

Figure 9.2. Accumulator column with separatory funnel.

(9) Nine Kuderna-Danish apparatus. The apparatus consists of:
   (a) Three-ball macro-Snyder columns (Kontes #K503000) - 300 mm length with 24/40 joints.
   (b) Evaporative flask (Kontes #K570002) - 500 mL with 24/40 top joint and 12/22 lower joint.
   (c) Concentrator tube (Kontes #K570000) - 10 mL graduated, with 19/22 joint. Attach to evaporative flask with springs (Kontes K503000-0232). Ground glass stopper (size 19/22 joints) is used to prevent evaporation of extracts.

(10) Nine modified micro-Snyder columns (Kontes #K569251) with 19/22 ground glass joint.

(11) One heated water bath or steam bath with concentric ring cover capable of temperature control. Bath should be located in a hood.

(12) One pH meter with a combination electrode.

(13) One manifold with temperature controlled water bath or aluminum heating block for nitrogen blowdown. A manifold with nine spaces is preferred but not essential.

(14) Pasteur pipettes.

(15) Twelve 1 dram glass vials with screw caps (Supelco 2-3213) and Teflon lined rubber septa (Supelco 2-3216).

(16) Volumetric flasks for preparing standard solutions - 10 mL.

(17) Syringes for preparing standard solutions - 50 and 250 μL.

(18) Graduated pipettes for preparing standard solutions - 1 mL.

(19) Thirty 15 mL glass vials with Teflon lined screw caps for storing standard solutions.

(20) One gas chromatograph suitable for capillary column chromatography with flame ionization detection and all required accessories including syringes, gases, and a strip chart recorders.

(21) One 30 m × 0.34 mm I.D. DB-1 (1.0 μ film thickness) fused silica capillary column.

(22) Materials and Reagents
   (a) Reagent water - reagent water is defined as a water source that does not produce a background interference at the limit of detection. A water purification system (Millipore

Super-Q or equivalent) may be used to generate reagent water.

(b) 1.5 L methanol (Burdick and Jackson, pesticide grade).

(c) 500 mL ethyl acetate (Burdick and Jackson, pesticide grade).

(d) 3 L methylene chloride (Burdick and Jackson, pesticide grade).

(e) 120 mL Amberlite XAD-4 macroreticular resin (mesh 20-60, Rohm and Haas, Philadelphia, Pennsylvania).

(f) 100 mL 0.1N H_2SO_4 in reagent water.

(g) 100 mL 0.1N NaOH in reagent water.

(h) 200 mL of 1.0 M pH 8.0 phosphate buffer. Weigh 74.4 g of K_2HPO_4 and 7.64 g of KH_2PO_4 in a 200 mL volumetric flask and dilute to the mark with reagent water.

(i) 10 mL $Na_2S_2O_3$ solution - 0.05 M in reagent water [use the same solution as specified in Chapter 4, section 4.2(14)(f)].

(j) 1 mL external standard solution - 4-fluoro-2-iodotoluene (300 ng/10 μL).

(k) 1 mL GC/FID external standard solution - hexadecane (30 ng/10 μL) in CH_2Cl_2.

(l) 10 mL system performance solution (Table 9.1).

9.3 PREPARATION FOR ANALYSIS

9.3.1 Preparation of WABN System Performance Solution

(1) Prepare individual stock standards (12 mg/mL).

- solids - accurately weigh 0.120 g of pure material, dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.
- liquids - with a 250 μL syringe accurately measure 120 μL of pure liquid, dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.

(2) Prepare a secondary standard. With a 1 mL graduated pipette accurately measure 0.25 mL of each stock standard into a 10 mL volumetric flask [for quinoline and 6-t-butyl-m-cresol use 0.8 mL, for 4-fluoro-2-iodotoluene use 125 μL (measure with a 250 μL syringe), for 2,4,6-trimethylpyridine and m-cresol use 25 μL (measure with a 50 μL syringe), and for methyl stearate use

Table 9.1. SYSTEM PERFORMANCE SOLUTION FOR WABN FRACTION[a]

Compound	Density (@ 20°C)	Concentration (ng/μL)
2,6-dimethylphenol	S[b]	300
2,6-dimethylaniline	0.984	300
acetophenone	1.030	310
1-tetradecanol	0.823	240
1-octadecene	0.789	240
n-octadecane	0.777	230
DFTPP	S	300
n-eicosane	S	300
pyrene	S	300
n-heneicosane	S	300
methyl stearate	S	10
d_{10}-*o*-xylene	0.897	270
d_8-naphthalene	S	300
d_5-nitrobenzene	1.204	360
d_5-phenylethanol	1.023	310
d_5-propiophenone	1.009	300
d_5-acetophenone	1.030	310
d_{12}-perylene	S	300
4-fluoro-2-iodotoluene	0.883	270
d_9-acridine	S	300
d_6-phenol	S	300
quinoline	1.095	1090
2,4,6-trimethylpyridine	0.917	23
m-cresol	1.034	26
6-*t*-butyl-*m*-cresol	S	1000

[a] See Table 9.11 for the function of each test component in the system performance evaluation.

[b] S = solid.

10 μL (measure with a 50 μL syringe)]. Dilute to volume using methylene chloride to give the concentrations in Table 9.1. Transfer into a Teflon sealed screw cap bottle for convenience in use. Store at 4°C.

(3) Transfer the stock standards into Teflon sealed screw cap bottles for convenience in use. Store at 4°C.

(4) Fresh standard should be prepared every six months. If degradation or evaporation has occurred, fresh standards should be prepared sooner.

(5) If compound purity is 96% or greater, the weight or volume can be used without correction to calculate the concentration of stock standards. If the compound is less than 96% pure, it cannot be used as a standard.

9.3.2 Preparation of Resin Material

Amberlite XAD-4 resin material is cleaned prior to preparing the resin columns. The minimum amount of resin that should be prepared is 120 mL which is the amount needed to process nine water samples plus two procedural blanks. The following instructions describe procedures based on 120 mL of resin material; however, it is possible to prepare larger batches and store the prepared resin under methanol in a sealed glass jar. If a larger volume of resin is prepared, volumes of cleaning solvents should be adjusted proportionately.

Place the resin material in a 500 mL Erlenmeyer flask. Make a slurry of the resin with 250 mL of distilled water. Stir the resin gently to avoid fracturing beads. After allowing the resin to settle for 30 seconds, remove fines by decantation. Repeat the slurry-decanatation procedure three times. Then rinse the resin three times with 250 mL portions of methanol. Place the resin in a Soxhlet extractor and extract with pesticide analysis grade ethyl acetate for eight to sixteen hours (cycle time: ~10 minutes). Then extract the resin with pesticide analysis grade methanol for an additional eight to sixteen hours (cycle time: ~10 minutes). Store cleaned resin in a sealed glass jar under methanol with a Teflon lined screw cap.

9.3.3 Cleaning of Materials

(1) Glassware to be used is washed with Amway S-A-8 laundry compound (or equivalent), rinsed with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with foil (precleaned with hexane) to prevent contamination.

(2) Teflon liners and teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. The sonicated liners are vacuum-oven (~20 inches of water) dried for 3 to 5 hours at 70° and stored in clean, Teflon lined screw cap bottles.

9.3.4 GC/FID Performance Evaluation

Prior to analyzing procedural blanks, acceptable performance for the GC/FID system must be demonstrated.

(1) Analyze the GC/MS performance standard (Table 9.1) as specified in Table 9.2. Figure 9.3 shows a total ion chromatogram of this mixture analyzed by GC/MS under similar conditions which may be used to identify test components in the standard.

(2) Measure peak asymmetry or tailing for acetophenone and 1-tetradecanol using the percent peak asymmetry factor (PAF):

$$\%\ \text{PAF} = \frac{B}{F} \times 100$$

where

B = the width of the back half of a chromatographic peak measured at 10% above baseline.

F = the width of the front half of a chromatographic peak measured at 10% above baseline.

PAF should measure less than 200% for both acetophenone and 1-tetradecanol.

(3) Check the acidity/basicity of the column by the peak area ratios determined by integrator or triangulation of 2,6-dimethylaniline and 2,6-dimethylphenol to acetophenone. A ratio of 0.7 to 1.3 for both is acceptable.

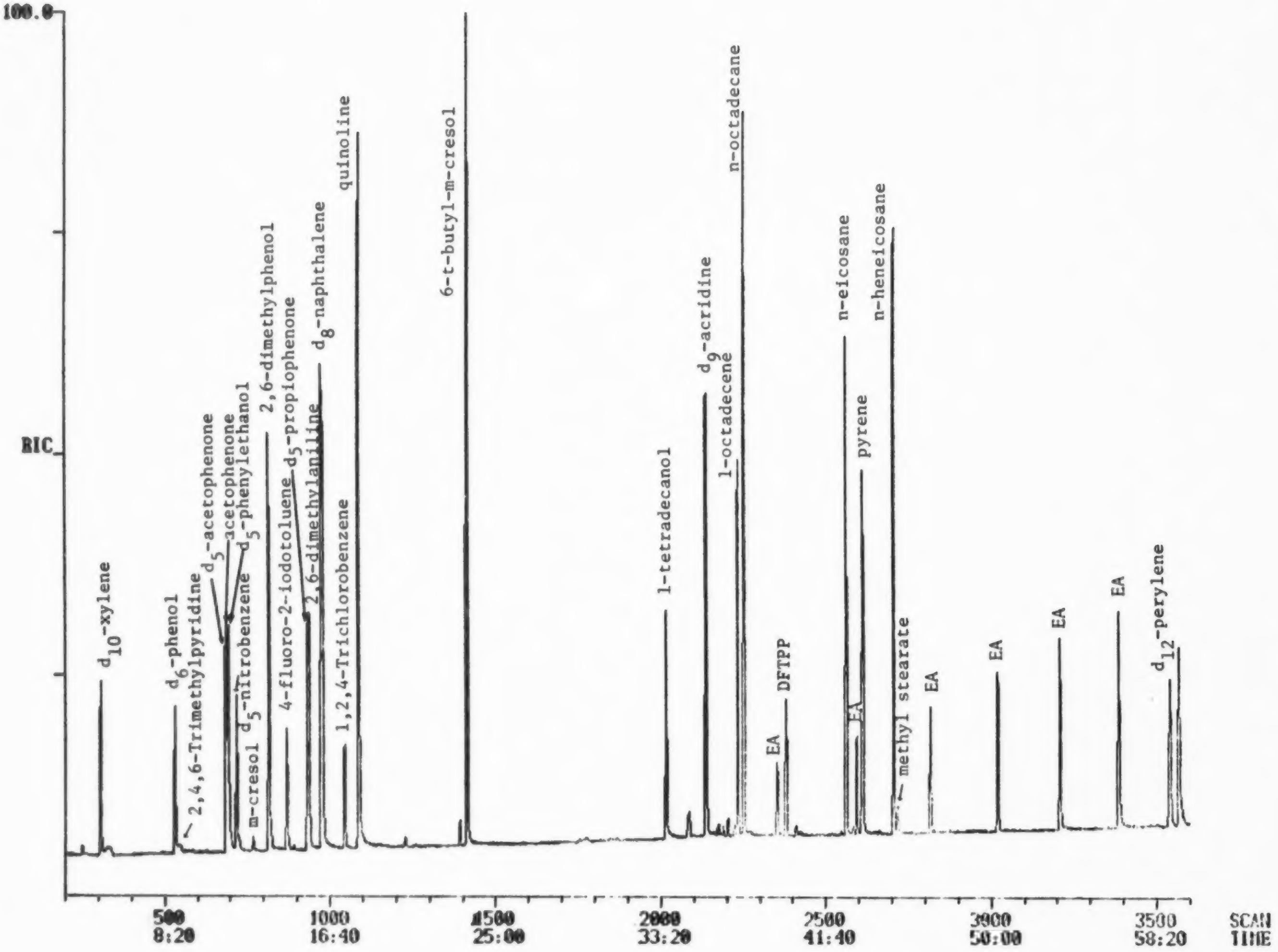


Figure 9.3. Total ion chromatogram of WABN SPS (EA = experimental analyte).

Table 9.2. GC/FID OPERATING CONDITIONS FOR EXTRACTABLE WEAK ACIDS, BASES AND NEUTRALS

GC Column	30 m DB-1 (1.0 μ film thickness) fused silica, wide bore (0.34 mm I.D.), capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through column; 15:1 split injection
Temperature Program	50°C/5 min to 250°C @ 4°/min
Injector Temperature	250°C
Detector Temperature	260°C
Injection Volume	1.0 μL

(4) Sensitivity is checked by measuring signal-to-noise ratios for 2,4,6-trimethylpyridine, m-cresol, and methyl stearate. A signal-to-noise ratio of 10 to 1, or more for all three compounds is acceptable.

9.3.5 Procedural Blanks

For each lot of materials and reagents that are used, a set of three procedural blanks are required. Table 9.3 identifies these blanks and defines their purpose. These blanks must be processed and analyzed by GC/FID prior to processing any samples. If a large number of samples are to be processed, it is advantageous to prepare large lots of materials and reagents thereby reducing the number of blanks which must be run. Procedural blank 1, however, must be run every time a new batch of samples is processed and analyzed.

9.3.5.1 Procedural Blank 1--

(1) Prepare a resin column as described in step 9.4.1, then follow the extraction procedure as described in steps 9.4.6 to 9.4.15.

(2) With a 50 μL syringe, add 10 μL of the GC external standard [9.2(22)(k)] to the blank concentrate. Agitate the K-D receiver to give a uniform distribution of standard in the concentrate.

(3) Analyze samples by GC/FID using the conditions described in Table 9.2. Contaminant peak heights should measure less than 20% relative to the internal standard.

Table 9.3. PROCEDURAL BLANKS

Blank	Description
Procedural Blank 1	detects contamination in resin material, solvents, and glassware
Procedural Blank 2	detects contamination in reagent water
Procedural Blank 3	detects contamination in sulfuric acid, sodium hydroxide, sodium thiosulfate, and phosphate buffer solutions used during sample collection and analysis

(4) If the blank is acceptable, add a 10 µL aliquot of the MS external standard solution [9.2(22)(j)] using a 50 µL syringe. Agitate the K-D receiver to give a uniform distribution of standard in the concentrate. Transfer a measured portion (0.3 to 0.4 mL) of the concentrate to a 1 dram vial and cap. Store at 0°C until GC/MS analysis.

(5) If significant contamination is present, the procedure should be repeated using a blank chromatography column with no resin added. If this blank is acceptable, then the resin is the source of contamination and should be recleaned using the procedure in Section 9.3.2. A procedural blank 1 should then be repeated using the fresh resin material.

(6) If significant contamination is present in this blank, fresh reagents should be used and the reagent blank (no resin) repeated. When the reagent blank is acceptable, a procedural blank 1 must be repeated. Procedural blanks 2 and 3 may be processed once the procedural blank 1 is acceptable.

9.3.5.2 Procedural Blanks 2 and 3--

(1) Prepare a resin column as described in step 9.4.1. Attach a separatory funnel to the top of the column. For procedural blank 2, pour 100 mL of reagent water into the separatory funnel, then drain the sample through the resin bed at a flow rate of ~10 mL/minute. For procedural blank 3 use 100 mL of reagent

water spiked with 1 mL each of the sulfuric acid, sodium hydroxide, and sodium thiosulfate solutions, and 10 mL of the phosphate buffer solution. The first three must be the same solutions which were used when the samples were collected (Chapter 4). See 9.2(22)(h) for phosphate buffer solution.

(2) Follow the extraction procedure as described in steps 9.4.6 to 9.4.15.

(3) With a 50 μL syringe, add 10 μL of the GC external standard [9.2(22)(k)] to the blank concentrates. Agitate the K-D receiver to give a uniform distribution of standard in the concentrates.

(4) Analyze the samples by GC/FID using the conditions in Table 9.2. Peak heights for contaminants in Procedural blank 3 which are not present in procedural blank 2 should measure less than 20% relative to the internal standard.

(5) If significant contamination is present in procedural blank 3 which is not present in procedural blank 2, the procedure should be repeated using fresh solutions of sulfuric acid, sodium hydroxide, sodium thiosulfate, and phosphate buffer until an acceptable blank is achieved.

9.4 SAMPLE EXTRACTION

(1) Prepare resin column by placing a small glass wool plug in the bottom of the chromatography column. Using a graduated pipette with the tip cut off, pipette 10 mL (measure volume after resin has settled) of cleaned XAD-4 resin into the open tubular chromatography columns, allow the resin to settle, and rinse the column with ~50 mL of reagent water.

(2) Add 10 mL of the phosphate buffer solution to the sample. Mix well. Check the pH of the sample using a pH meter. Adjust the sample to 8 using 0.1N H_2SO_4 or 0.1N NaOH.

(3) Attach siphoning adaptor (Figure 9.1) between sample containers and resin column. Attach a piece of tygon tubing between the end of the chromatography column and a water aspirator. To start sample flowing through the resin, open column stopcock and turn on aspirator. This should create sufficient suction to

pull the water sample through the siphon apparatus onto the resin column. Once flow starts disconnect the aspirator.

(4) Using the stopcock at the base of the column, adjust flow through the column to ~10 mL/minute. At this flow it should take ~18 to 20 hours to pass the sample through the resin bed. Sample may be discarded after elution through the column.

(5) After the sample has passed through the column, remove the siphon adaptor from the column. If any sample remains in the containers, transfer it to the separatory funnel, attach the funnel to the resin column as illustrated in Figure 9.2, and pass remaining sample through the resin bed.

(6) Once all sample has passed through the column, remove the separatory funnel and attach a piece of tygon tubing between the end of the column and a water aspirator. Aspirate any remaining water off the column (aspirate for 2 to 3 minutes). It is important to remove all water during this procedure.

(7) Add 12 mL of methanol to the dried resin bed. Stopper the column and shake to remove any air bubbles. Equilibrate the column for 10 minutes.

(8) Rinse the containers for each sample with a total of 200 mL methylene chloride. Pour the methylene chloride rinse into the separatory funnel.

(9) Drain all of the methanol from the resin bed. Collect the eluate in a 500 mL glass bottle with a Teflon lined screw cap.

(10) Add ~15 mL methylene chloride to the resin bed, allow air bubbles to escape. Place a glass wool plug on the top of the resin bed.

(11) Collect ~5 mL of methylene chloride from the separatory funnel, then return to the separatory funnel. This removes any entrained water from the stopcock and connector joint.

(12) Attach the separatory funnel to the column. Elute the column with the methylene chloride. Stop eluting when the methylene chloride reaches the top of the resin bed. Do not elute any water through the resin. Collect eluant in the 500 mL bottle containing the methanol rinse. Eluant may be stored at 0°C after this operation.

(13) Transfer the eluant to a 500 mL K-D flask equipped with a 10 mL concentrator tube containing several glass boiling beads. Precalibrate the concentrator tube to compensate for the volume of the glass boiling beads. Rinse the bottle with three 5 mL portions of methylene chloride and transfer these rinses to the K-D flask. Attach the three-ball macro-Snyder column. Place the K-D apparatus on a warm water bath with the concentrator tube partially immersed in water or on a steam bath. Adjust the temperature of the water bath such that evaporating solvent causes the balls in the Snyder column to chatter actively but does not flood the chambers of the column. Under these conditions evaporation of 500 mL of methylene chloride to 2 mL should take 15 to 20 minutes.

(14) When the liquid has reached an apparent volume of 2 mL remove the K-D apparatus from the heat and allow to cool. Remove the Snyder column. With 1 to 2 mL of solvent, rinse the evaporation flask and its lower joint into the concentrator tube. The final volume in the concentrator tube should be approximately 4 mL.

(15) Attach a modified micro-Snyder column to the K-D receiver. Connect a transfer pipette to the nitogen manifold using Teflon tubing. Place the pipette in the modified Snyder column above the solvent level (Figure 9.4). Gently blow a stream of nitrogen above the solvent surface until the volume is reduced to approximately 1 mL (CAUTION: contamination can occur if tygon tubing is used to connect the manifold to the nitrogen source). Rinse the sides of the concentrator tube with approximately 0.5 mL of solvent. Reduce volume of eluant to 0.5 mL using nitrogen blowdown. Record final volume.

(16) With a 50 µL syringe, add 10 µL of MS external standard solution to the sample concentrate. Agitate the K-D receiver to give an uniform distribution of external standard in the sample concentrate. Transfer a measured portion (0.3 to 0.4 mL) of the sample concentrate to a 1 dram vial and cap. Store at 0°C until GC/MS analysis.

(17) Proceed to Section E - GC/MS/COMP ANALYSIS OF WABN-SC, WABN-BL, or WABN-FU SAMPLE EXTRACTS.

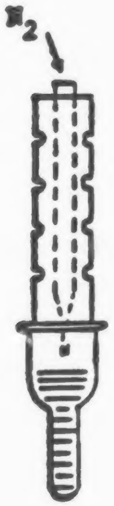


Figure 9.4. Nitrogen blowdown with modified micro-Snyder column.

B. BATCH LIQUID-LIQUID EXTRACTION (WABN-BL)

9.5 INTRODUCTION

9.5.1 Principle of the Method

Batch liquid-liquid extraction is used to extract and concentrate weak acids, some bases, and neutral (WABN) organic compounds from aqueous samples that are not prone to emulsion formation, including surface waters and some effluents and hazardous waste leachates. The 1 L sample is collected in a 1 quart bottle with 60 mL of a keeper solvent (35% methylene chloride in hexane). The extraction is performed by first transferring the keeper solvent and sample to a 2 L separatory funnel, draining the sample back into the sample bottle, transferring the keeper solvent to a 1 L bottle, and returning the aqueous sample to the separatory funnel. The pH of the aqueous sample is adjusted to 8.0 and the sample extracted with three 200 mL portions of methylene chloride. The methylene chloride extracts are combined with the keeper solvent, dried by passing through an anhydrous sodium sulfate column and evaporated to ~4 mL using a macro Kuderna-Danish evaporator and further concentrated to 0.5-1.0 mL by nitrogen blowdown using a modified micro-Snyder column. The concentrated extract is screened to determine if clean-up will be required by packed column gas chromatography/flame ionization detection. If clean-up is not required the extract is analyzed by capillary column gas chromatography/mass spectrometry, otherwise the protocol for sample clean-up is to be implemented.

9.5.2 Detection Limit and Sample Size

Since all water types covered by this protocol have the same initial sample size, detection limits will depend upon the final volume of sample concentrate. If the detection limit for GC/MS analysis of each extractable organic is estimated at 10 ng and samples are concentrated from an original volume of 1 L, then using a 1 μL injection size, nominal detection limits for specific water types are as follows:

5 ppb in surface waters (0.5 mL final volume)

10 ppb in industrial and municipal wastewaters, and energy effluents (1.0 mL final volume)

If the sample cannot be concentrated to the specified volume due to high levels of organic components, nominal detection limits are proportionately higher.

9.5.3 Interferences

A clean-up fractionation procedure is specified which is implemented when the chromatographable organics are present in high levels and would interfere with GC/MS analysis. A procedural blank must be run prior to processing any samples to assure that contamination is low.

9.5.4 Precision, Accuracy, and Scope

Table 1.5 in Chapter 1 presents average recovery and standard deviations for a variety of extractable compounds.

9.6 APPARATUS AND REAGENTS

The following materials are required for processing a set of nine samples plus two procedural blanks. Nine is the maximum number of samples which should be processed at a time. The quality control samples that should be run during these analyses are listed in Table 4.1 of Chapter 4. The two procedural blanks should be run before any samples are collected or processed. The extraction/concentration procedure will require two working days for a set of nine samples.

(1) Nine separatory funnels (2000 mL) with Teflon stopcocks.

(2) Nine 1 L amber glass bottles with Teflon-lined screw caps for collecting the extracting solvent prior to solvent evaporation.

(3) One 1 L graduated cylinder for measuring sample volumes.

(4) Nine chromatographic columns for sodium sulfate solvent drying. 300 mm × 10 mm i.d. with a glass wool plug at the bottom, containing about 85 g Na_2SO_4 (Fig. 9.5).

(5) One Soxhlet extractor (50 mL capacity minimum) for extracting glass wool.

(6) Eighteen glass funnels to fit chromatography columns and separatory funnels.

(7) Pre-extracted glass wool (~50 mL) for drying columns. Glass wool should be precleaned by extracting in a Soxhlet overnight with methylene chloride.

(8) Boiling chips - Hengar granules are ground with a mortar and pestle and sieved to obtain a 60/80 mesh. These granules are

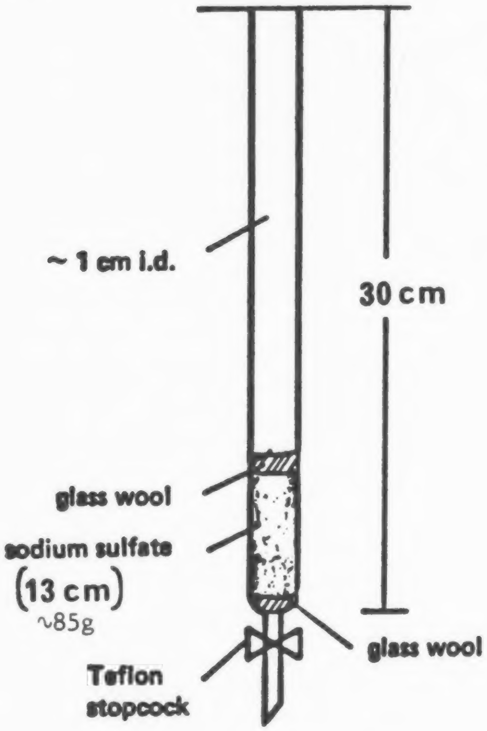

~ 1 cm i.d.
30 cm
glass wool
sodium sulfate
(13 cm)
~85g
glass wool
Teflon
stopcock

Figure 9.5. Sodium sulfate drying column.

cleaned by extracting ~5 g three times with ~150 mL methylene chloride.

(9) Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

(10) Balance - analytical, capable of accurately weighing to the nearest 0.0001 g.

(11) One pH meter.

(12) One manifold with a temperature controlled water bath for nitrogen blowdown. A manifold with nine spaces is preferred but not essential.

(13) Pasteur pipettes.

(14) One 5 mL pipette for spiking water samples with internal standard solution.

(15) Fifteen glass vials (1 dram) with 13 mm screw caps (Supelco 2-3213) and Teflon lined rubber septa (Supelco 2-3216).

(16) One gas chromatography column (180 cm × 2 mm i.d.) packed with 3% OV-1 on Chromosorb W HP (80/100 mesh) or equivalent.

(17) One gas chromatograph with flame ionization detector and strip chart recorder.

(18) Five ring stands or ring supports suitable for supporting a 2000 mL separatory funnel.

(19) Counter top centrifuge capable of ~4000 rpm.

(20) Nine 10-15 mL centrifuge tubes with screw caps.

(21) Ten mL volumetric flasks for preparing standard solutions.

(22) Syringes for preparing standard solutions.

(23) Graduated pipettes for preparing standard solutions.

(24) Thirty 15 mL glass vials with Teflon lined screw caps for storing standard solutions.

(25) One gas chromatograph suitable for capillary column chromatography with flame ionization detection and all required accessories including syringes, gases, and a strip chart recorders.

(26) One 30 m × 0.34 mm I.D. DB-1 (1.0 μ film thickness) fused silica capillary column.

(27) Nine Kuderna-Danish apparatus [see 9.2(9)].

(28) Materials and Reagents

   (a) 6 L methylene chloride (Burdick and Jackson, distilled in glass).

   (b) 100 mL H_2SO_4 solution (1 + 1). Add 50 mL of concentrated H_2SO_4 (sp. gr. 1.84) slowly to 50 mL of reagent water.

   (c) 100 mL 6N NaOH. Weigh 24 g of NaOH into a 100 mL volumetric flask and dilute to the mark with reagent water.

   (d) 1000 g anhydrous sodium sulfate powder.

   (e) 1 mL external standard solution: 4-fluoro-2-iodotoluene (30 mg) and 2-fluorobiphenyl (30 mg) in 10 mL CH_2Cl_2.

   (f) Alkane calibration standard - dissolve 50 mg each of n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane, and n-heptadecane in 10 mL methylene chloride.

(g) 1 mL WABN system performance solution (Table 9.1).

(h) 90 mL of 1.0 M pH 8.0 phosphate buffer. Weigh 15.0 g of K_2HPO_4 and 1.91 g of KH_2PO_4 in a 100 mL volumetric flask and dilute to the mark with reagent water.

(i) Reagent water - reagent water is defined as a water source which does not produce a background interference at the limit of detection. A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

9.7 PREPARATION FOR ANALYSIS

9.7.1 Preparation of WABN System Performance Solution

(1) Prepare individual stock standards (12 mg/mL) of the compounds listed in Table 9.1.

- solids - accurately weigh 0.120 g of pure material, dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.
- liquids - with a 250 μL syringe accurately measure 120 μL of pure liquid; dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.

(2) Prepare secondary standard with a 1 mL graduated pipette accurately measure 0.25 mL of each stock standard into a 10 mL volumetric flask (for quinoline and 6-*t*-butyl-*m*-cresol use 0.8 mL, for 4-fluoro-2-iodotoluene use 125 μL [measure with a 250 μL syringe], for 2,4,6- trimethylpyridine and *m*-cresol use 25 μL [measure with a 50 μL syringe], and for methyl stearate use 10 μL [measure with a 50 μL syringe]). Dilute to volume using methylene chloride to give the concentrations in Table 9.1. Transfer into a Teflon sealed screw cap bottle. Store at 4°C. Fresh standard should be prepared every six months. If degradation or evaporation has occurred, fresh standards should be prepared sooner.

(3) If compound purity is 96% or greater, the weight or volume can be used without correction to calculate the concentration of stock standards. If the compound is less than 96% pure, it cannot be used as a standard. Transfer the stock standards into Teflon sealed screw cap bottles for convenience. Store at 4°C.

9.7.2 Cleaning of Materials

(1) Glass glassware to be used is washed with Amway S-A-8 laundry compound (or equivalent) rinsed with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with foil to prevent contamination.

(2) Teflon liners and Teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. The sonicated liners are vacuum-oven (~20 inches of water) dried for 3 to 5 hours at 70° and stored in clean, Teflon lined screw cap bottles.

(3) Anhydrous sodium sulfate is cleaned and dried prior to use. Approximately 1000 g of material is needed to process eight samples and is the minimum amount of material which should be prepared. The following instructions describe procedures based on 1000 g of material; however, it is possible to prepare larger batches and store the prepared material in a sealed Erlenmeyer flask. If a larger quantity of material is prepared, volumes for rinsing should be adjusted accordingly.

Sodium sulfate (1000 g) is placed in a 1 L Erlenmeyer flask with a ground glass joint. Methylene chloride (600 mL) is added, the flask is swirled for 5 minutes, and the solvent decanted. The procedure is repeated two additional times. To dry the sodium sulfate, the cleaned material is heated in the flask in a drying oven at 130°C overnight. The clean dried material can be stored in the drying oven, or stoppered and stored in a dessicator.

To prepare drying columns, a small piece of pre-extracted glass wool is placed in the bottom of the chromatography column, 13 cm of sodium sulfate is poured into the column, followed by an additional plug of pre-extracted glass wool. Columns should be used immediately or stored in a drying oven at 130°C. Rinse the Na_2SO_4 with 50 mL of extracting solvent just prior to using the column.

(4) Glass wool is cleaned by placing in the extraction chamber of a Soxhlet extractor and extracting overnight with distilled in glass methylene chloride. The glass wool is placed in a wide mouth jar and the solvent removed either under nitrogen stream or by warming. Cap and store until needed.

9.7.3 GC/FID Performance Evaluation

Prior to analyzing procedural blanks, acceptable performance for the GC/FID system must be demonstrated.

(1) Analyze the GC/MS performance standard (Table 9.1) as specified in Table 9.2. Figure 9.3, (Part I), shows a total ion chromatogram of this mixture analyzed by GC/MS under similar conditions, which may be used to identify test components in the standard.

(2) Measure peak asymmetry or tailing for acetophenone and 1-tetradecanol using the percent peak asymmetry factor (PAF):

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

where

B = the width of the back of a chromatographic peak to the perpendicular from the peak measured at 10% above baseline.

F = The width of the front of the chromatographic peak to the perpendicular from the peak measured at 10% above baseline.

the PAF should measure less than 200% for both acetophenone and 1-tetradecanol.

(3) Check the acidity/basicity of the column by the peak area ratios determined by integrator or triangulation of 2,6-dimethylaniline and 2,6-dimethylphenol to acetophenone. A ratio of 0.7 to 1.3 for both is acceptable.

(4) Sensitivity is checked by measuring signal to noise ratios for 2,4,6-trimethylpyridine, m-cresol, and methyl stearate. A signal-to-noise ratio of 10 or greater for all three compounds is acceptable.

9.7.4 Procedural Blanks

For each lot of materials and reagents that are used, a set of three procedural blanks are required. Table 9.4 identifies these blanks and defines their purpose. These blanks must be processed and analyzed by GC/FID prior to processing any samples. If a large number of samples are to be processed, it is advantageous to prepare large lots of materials and reagents thereby reducing the number of blanks which must be run. Procedural Blank 1, however, must also be run every time a new batch of samples is processed and analyzed.

9.7.4.1 Procedural Blank 1--

(1) Rinse separatory funnel with keeper solvent (60 mL of 35% methylene chloride in hexane) and drain into 1 L amber glass bottle. Follow the extraction procedure as described in steps 4.2.4 to 4.2.14. (Use same lots of the keeper solvent as used during sample collection.)

(2) Add 10 μL of the GC external standard to the blank concentrate. Agitate the K-D receiver to give a uniform distribution of standard in the concentrate.

(3) Analyze samples by GC/FID using the conditions described in Table 9.2. Contaminant peak heights should measure less than 20% relative to the external standard.

(4) If procedural blank 1 is acceptable, add an aliquot (10 μL) of the MS external standard solution. Agitate the K-D receiver to give a uniform distribution of standard in the concentrate.

Table 9.4. PROCEDURAL BLANKS

Blank	Description
Procedural Blank 1	detects contamination in solvents and glassware
Procedural Blank 2	detects contamination in reagent water
Procedural Blank 3	detects contamination in sulfuric acid, sodium hydroxide, sodium thiosulfate, and phosphate buffer solutions used during sample collection and analysis

Transfer the concentrate to a 1 dram vial and cap. Store at 0°C until GC/MS analysis.

(5) If significant contamination is present, the procedure should be repeated using freshly cleaned glassware.

(6) If significant contamination is still present in this blank, fresh reagents (different lots or sources of solvents) should be used. When procedural blank 1 is acceptable, procedural blanks 2 and 3 may be processed.

9.7.4.2 Procedural Blanks 2 and 3--

(1) Procedural blank 2 is prepared from reagent water (920 mL) placed in a sample bottle. Cover with 60 mL of "keeper solvent" (35% methylene chloride:65% hexane). Procedural blank 3 is prepared in the same way as 2 except that the reagent water is spiked with 1 mL each of the surfuric acid, sodium hydroxide, and sodium thiosulfate to be used for sample collection (see Chapter 4), and 10 mL of phosphate buffer [9.2(27)(i)].

(2) Follow the extraction procedure as described in steps 9.8.2(2) to 9.8.2(14).

(3) Add 10 µL of the GC external standard to the blank concentrate. Agitate the K-D receiver to give a uniform distribution of standard in the concentrate.

(4) Analyze the samples by GC/FID using the conditions in Table 9.2. Peak heights for contaminants in procedural blank 3 which are not present in procedural blank 2 should measure less than 20% relative to the external standard.

(5) If significant contamination is present in procedural blank 3 which is not present in procedural blank 2, the procedure should be repeated using fresh solutions of sulfuric acid, sodium hydroxide, sodium thiosulfate, and phosphate buffer until an acceptable blank is achieved.

9.8 SAMPLE EXTRACTION

9.8.1 Determination of Emulsion Prone Samples

All water samples except drinking water must be screened prior to processing to separate emulsion prone samples from samples which can adequately be extracted using the separatory funnel method. The scouting sample (5 mL) is placed in a 10 mL glass centrifuge tube with screw cap and the sample pH adjusted to pH 8 with 6N NaOH or 1:1 H_2SO_4. Methylene chloride (2 mL) is added, the vial sealed and the mixture shaken for two minutes (~60 cycles per minute). If the solvent and aqueous sample separate into two discrete layers in less than 15 minutes then the separatory funnel extraction may be used without modification. If two layers do not form, centrifuge for 1 minute at ~4000 rpm and inspect again. If two layers have formed, mark samples as slightly emulsion prone. If no separation of layers occurs then use the flow-under extractor designed for emulsion prone samples (Chapter 9, Part C).

9.8.2 Sample Extraction

(1) Mark the interface between the "keeper" solvent and water on the sample bottle with an indelible marker.

(2) Transfer the entire contents of the sample bottle into a 2000 mL separatory funnel and drain the lower aqueous layer back into the sample bottle. Tap the separatory funnel several times to remove water drops adhering to the sides. When all of the water has been removed drain the solvent into a 1 L amber glass bottle through a Na_2SO_4 drying column.

(3) Return the sample to the separatory funnel and adjust the pH to 8.0 using 6N NaOH or 1:1 H_2SO_4. Check the pH with narrow range pH paper. Add 10 mL of 1.0 M pH 8.0 phosphate buffer.

(4) Rinse the sample bottle with 200 mL of methylene chloride and transfer to the separatory funnel.

(5) Shake the separatory funnel at ~120 cpm for 2 minutes, venting as required. For samples which are slightly emulsion prone shake gently (30-45 cpm) for 5 minutes.

(6) Return separatory funnel to ring stand and allow phases to separate for 30 minutes. Proceed with the extraction of other samples (steps 1-6). Four or five samples can be processed through this sequence with maximum utilization of time.

(7) Drain the methylene chloride through the same drying column (step 2) into the 1 L bottle, combining it with the "keeper" solvent.

(8) Add 200 mL of methylene chloride to the separatory funnel and extract again as described in steps 5-7. Repeat for a third extraction.

(9) Rinse Na_2SO_4 drying column with 25 mL of methylene chloride to complete extract transfer.

(10) Transfer ~1/2 the dried extract to a 500 mL K-D flask equipped with 4 mL concentrator tube containing several Hengar granules. The concentrator tube should be precalibrated to compensate for the volume of the granules. Attach the three-ball macro-Snyder column. Place the K-D apparatus on a warm water bath with the concentrator tube partially immersed in water, or on a steam bath. Adjust the temperature of the water bath such that evaporating solvent causes the balls in the Snyder column to chatter actively but does not flood the chambers of the column. Under these conditions evaporation of 500 mL of methylene chloride to 2 mL should take 15 to 20 minutes.

(11) When the liquid has reached an apparent volume of 2 mL remove the K-D apparatus from the heat and allow to cool. Remove the Snyder column.

(12) Transfer the remaining extract to the K-D apparatus and repeat steps 10 and 11.

(13) With 1 to 2 mL of solvent, rinse the evaporation flask and its lower joint into the concentrator tube. The final volume in the concentrator tube should be approximately 4 mL.

(14) Attach a modified micro-Snyder column to the K-D receiver. Connect a transfer pipette by Teflon tubing to a nitrogen manifold. Place the transfer pipette in the modified Snyder column above the liquid level (Figure 9.4). Gently blow a stream of nitrogen above the liquid surface until the volume is reduced to approximately 1 mL. Rinse the sides of the concentrator tube with approximately 0.5 mL of solvent. Reduce volume of extract to 0.5 for surface waters and to 1.0 mL for all other water types. Record final volume.

(15) To determine whether the extract is so complex that it must be cleaned up, a 1 μL injection is made on a gas chromatograph with

FID detection. The column (180 cm × 2 mm i.d.) is packed with 3% OV-1 on Chromosorb W HP. A temperature program from 30°C to 250°C at 10°/min is used. The attenuation is set to give 30 to 70% of full scale deflection for the various peaks of the alkane calibration standard.

(16) The chromatogram from step 15 is evaluated as follows: The baseline rise (A) is estimated by connecting the valleys of the chromatogram as shown in Figure 9.6. Draw a zero line (B) horizontal to t the level of the baseline before injection. At any point after the first 2 minutes of the chromatogram where A is more than 5% of full scale above line B, begin an incremental integration of the area between A and B. This is repeated in 5% increments as indicated by the shaded areas in Figure 9.6. Compute the area between A and B which is shaded (Area A-B). Measure the area of the peaks produced by the alkane calibration standard (Area C) by planimeter and compute the mg/mL equivalent of the baseline rise M_A by

$$\frac{M_A}{A_{A-B}} = \frac{40 \text{ mg/mL}}{A_C}$$

$$M_A = \frac{\text{Area}_{A-B} \times 40 \text{ mg/mL}}{\text{Area}_C}$$

A sample must be fractionated if $M_A > 50$ mg/mL (go to Section D). Otherwise it may be analyzed by GC/MS without further workup.

(17) Add 25 µL of MS external standard solution to the sample concentrate. Agitate the K-D receiver to give an uniform distribution of external standard in the sample concentrate. Transfer a measured portion of the sample concentrate to a one dram vial and cap. Store at 0°C until GC/MS analysis.

(18) Proceed to Section E - GC/MS/COMP ANALYSIS OF WABN-SC, WABN-BL, or WABN-FU SAMPLE EXTRACTS.

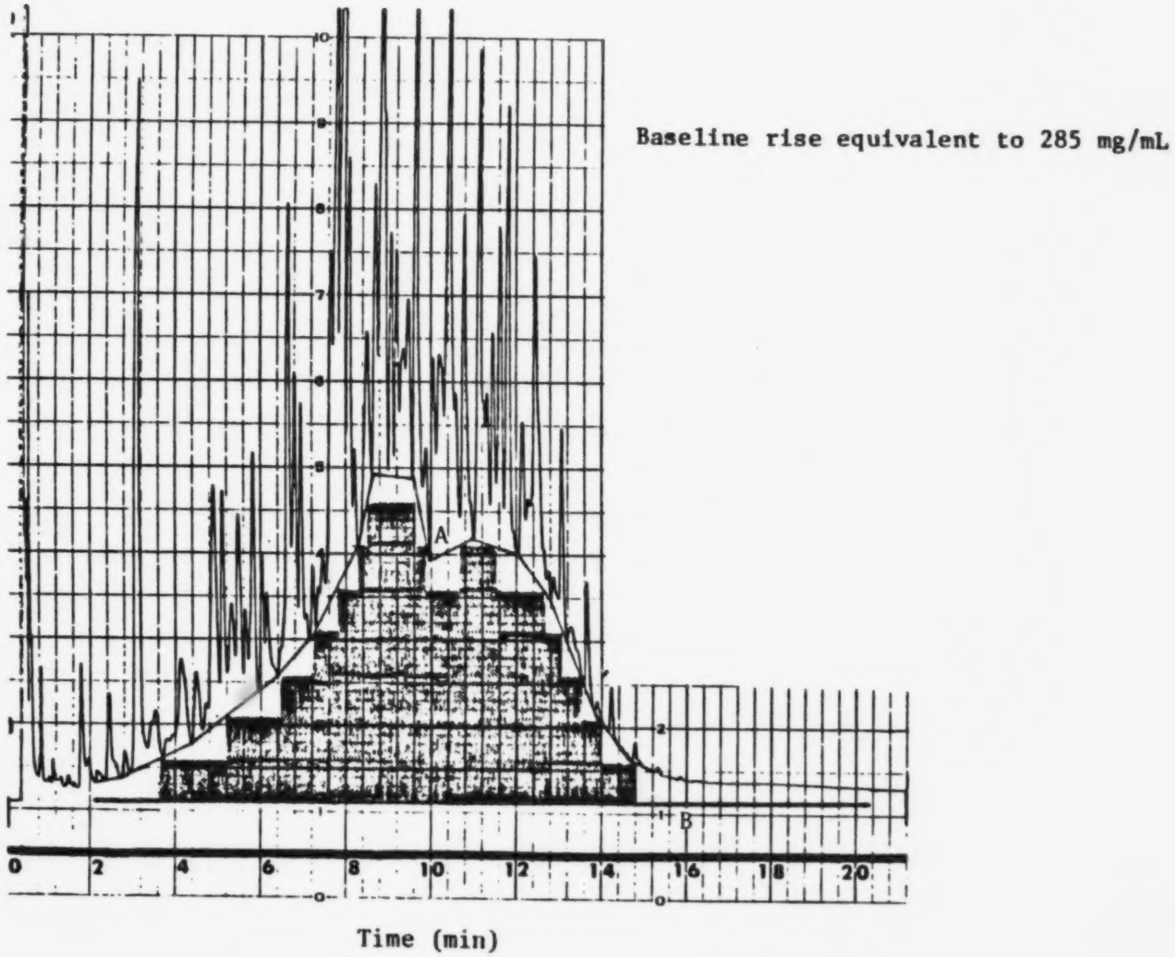


Figure 9.6. OV-1 packed column gas chromatogram of extract No. 8320 from a timber industry wastewater (see text for conditions).

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C. CONTINUOUS LIQUID-LIQUID EXTRACTION WITH FLOW-UNDER EXTRACTOR (WABN-FU)

9.9 INTRODUCTION

9.9.1 Principle of the Method

Weak acids, some bases, and neutral organic compounds are extracted and concentrated from emulsion prone aqueous samples by using a continuous liquid-liquid extraction technique with a flow-under extractor. (See Part B, Section 9.8.1 for determination of emulsion prone samples.) Because the flow-under extractor does not break the sample solvent interface, it prevents the formation of emulsion. The composition of emulsion prone wastewaters is extremely variable and these samples are subject to much greater variations in recoveries due to matrix effects than are wastewaters in general. For this reason the emulsion prone sample must be analyzed in duplicate, with one of the samples spiked with the surrogate compounds to determine recoveries.

The two samples are collected in 1 quart bottles and spiked with internal standards; 60 mL of a keeper solvent (35% methylene chloride in hexane) is then added and the sample shaken.

When ready for analysis, the keeper solvent is removed from the top of the bottle using a transfer pipet. The samples are transferred to two continuous liquid-liquid extractors (CLLE), adjusted to pH 8.0, and extracted with 500 mL of methylene chloride. After 3.5 hr of operation, the methylene chloride is removed, combined with the keeper solvent, dried over sodium sulfate, evaporated to a volume of 4 mL using Kuderna-Danish evaporation techniques and further concentrated to 0.5 to 1.0 mL by nitrogen blowdown using a modified micro-Synder column. The extracts are evaluated by packed column GC/FID to determine if fractionation is required. The concentrated extracts are analyzed by capillary column gas chromatography/mass spectrometry.

9.9.2 Detection Limit and Sample Size

Since all water types covered by this protocol have the same initial sample size, detection limit will depend upon the final volume of sample concentrates. If the detection limit for GC/MS analysis is estimated at 10 ng total material for extractable organics and samples are concentrated to 1.0 mL from an original volume of 1 L, then, using a 1 μL injection size, the nominal detection limit for emulsion prone wastewaters is 10 ppb. If the sample cannot be concentrated to the specified volume due to high levels of organic components, nominal detection limits are proportionately higher.

9.9.3 Interferences

A clean-up fractionation procedure is specified that is implemented when the chromatographable organics are present in high levels and would interfere with GC/MS analysis. A procedural blank must be run prior to processing samples to assure that contamination is low.

9.9.4 Precision, Accuracy, and Scope

The precision and accuracy of this method are highly dependent upon the particular matrix, so recoveries were determined during MAS development. Recovery and reproducibility must be estimated for each sample matrix through the use of surrogates and internal standards.

9.10 APPARATUS AND REAGENTS

The following materials are required for processing a set of four samples (2 water samples and 2 surrogate spiked samples; however, only one surrogate is required for each different sample matrix). Every set of 14 samples (7 water samples and 7 surrogate spiked samples) must contain an appropriate number of procedural blanks and procedural controls (Chapter 4, Table 4.1). The extraction/concentration procedure will require one working day for four samples. The limiting equipment will probably be the flow-under extractor. All other equipment and materials should be scaled proportionately.

(1) Four flow-under extractors (RTI Extractor Schematic, Figure 9.7).
(2) Four mechanical stirrers (Eberback Model 58 or T-Line Model 101-Talboys Engineering Company).
(3) Four heating mantles to hold a 500 mL round bottom flask.
(4) Four variable transformers.
(5) Four Friedrichs condensors (Corning 2640/with 24/40 inner joint at bottom with drip tip and 24/40 female joint at vapor outlet).
(6) One 1 L graduated cylinder for measuring sample volume.
(7) Four 1 L Erylenmeyer flasks.
(8) Four 250 mL Erlenmeyer flasks.

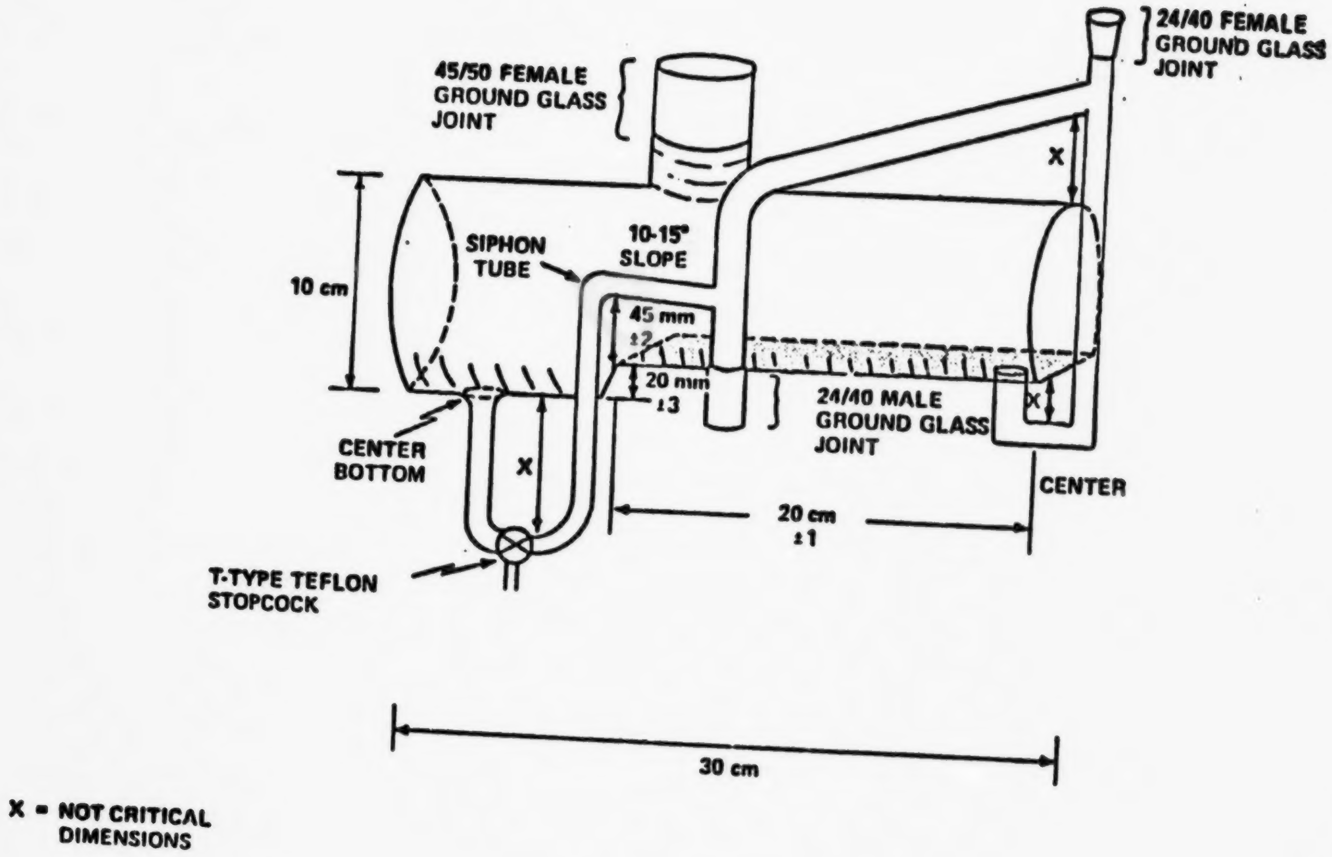

24/40 FEMALE GROUND GLASS JOINT
45/50 FEMALE GROUND GLASS JOINT
X
10 cm
SIPHON TUBE
10-15° SLOPE
45 mm ±2
20 mm ±3
24/40 MALE GROUND GLASS JOINT
X
CENTER BOTTOM
X
CENTER
20 cm ±1
T-TYPE TEFLON STOPCOCK
30 cm
X = NOT CRITICAL DIMENSIONS

Figure 9.7. RTI extractor schematic.

(9) Four long stem glass funnels.

(10) One 250 mL Erlenmeyer with ground glass joint.

(11) Four glass columns for sodium sulfate drying. Exact dimensions are not critical; however, mass of sodium sulfate should be approximately the same. Approximate dimensions are shown in Figure 9.5.

(12) Four glass funnels to fit drying columns.

(13) Glass wool (~30 mL) for drying columns. Glass wool should be precleaned by Soxhlet extracting overnight with methylene chloride.

(14) Boiling chips - Hengar granules are ground with a mortar and pestle and sieved to obtain a 60/80 mesh. These granules are cleaned by extracting ~5 g three times with ~150 mL methylene chloride.

(15) One Soxhlet extractor for cleaning glass wool. A minimum 50 mL capacity is required. Glass wool can be processed in larger batches and stored; therefore, a larger extractor is preferred.

(16) Four Kuderna-Danish apparatus. Each apparatus consists of
  (a) Three-ball macro-Snyder columns (Kontes # K503000) - 300 mm length with 24/40 joints,
  (b) Evaporative flask (Kontes # K570002 - 500 mL with 24/40 top joint and 19/22 lower joint,
  (c) Concentrator tube (Kontes # K570000) - 4 mL graduated, with 19/22 joint. Attach to evaporative flask with springs (Kontes K503000-0232. Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

(17) Four modified micro-Synder columns (Kontes # K569251) with 19/22 joint.

(18) One heated water bath or steam bath with concentric ring cover capable of temperature control. Bath should be located in a hood.

(19) One pH meter.

(20) One manifold with a temperature controlled water bath or temperature-controlled aluminum block for nitrogen blowdown. A manifold with eight spaces is preferred but not essential.

(21) Pasteur pipettes.

(22) Six 5 mL pipettes for spiking water samples with surrogate compound standard solution.

(23) Four 1 dram glass vials with screw caps (Supelco 2-3213) and Teflon lined rubber septa (Supelco 2-3216).

(24) One gas chromatograph suitable for capillary column chromatography with flame ionization detection and all required accessories including syringes, gases, and a strip chart recorder.

(25) One 30 m × 0.34 mm I.D. DB-1 (1.0 μ film thickness) fused silica capillary column.

(26) One gas chromatograph for packed column chromatography with flame ionization detection and all required accessories including syringes, gases, and a strip chart recorder.

(27) One 180 × 0.2 cm i.d. glass gas chromatography column packed with 3% OV-1 on Chromosorb W HP (100/120 mesh) or equivalent.

(28) Materials and Reagents

(a) 3 L methylene chloride (Burdick and Jackson, distilled in glass).

(b) 100 mL H_2SO_4 solution (1 + 1). Add 50 mL of concentrated H_2SO_4 (sp. gr. 1.84) slowly to 50 mL of reagent water.

(c) 100 mL 6N NaOH. Weigh 24 g of NaOH into a 100 mL volumetric flask and dilute to the mark with reagent water.

(d) 50 g sodium sulfate, anhydrous powder.

(e) Internal standard solutions (Table 4.3, Chapter 4, or if NBS Ampoules are available, Table 4.7, Chapter 4).

(f) 1 mL MS external standard solution: 4-fluoro-2-iodotoluene (30 mg).

(g) 5 mL surrogate standard solution (Table 9.5).

(h) 1 mL WABN system performance solution (Table 9.1).

(i) 4 mL of 1.0 M pH 8.0 phosphate buffer. Weigh 15.0 g of K_2HPO_4 and 1.91 g of KH_2PO_4 in a 100 mL volumetric flask and dilute to the mark with reagent water.

(j) Reagent water - reagent water is defined as a water source which does not produce a background interference at the limit of detection. A water purification system (Millipore

Table 9.5. SURROGATE STANDARD SOLUTION

Compounds[a]

Pyridine
α-Picoline
2-Heptanone
t-Butyl carbamate
2-*n*-Butoxyethanol
t-Butylpropionate
Benzaldehyde
Aniline
Lutidine
2-Octanone
Benzyl chloride
2,3,6-Trimethylpyridine
n-Decane
Salicylaldehyde
Phenyl acetate
Tolualdehyde
2,3-Dihydrobenzofuran
p-Cresol
Fenchone
3-Chlorobenzaldehyde
Isophorone
2-Bromo-1-chlorobenzene
o-Chloroanisole
4-Chlorobenzonitrile
1,4-Dicyanobutane
Di-*t*-butyldisulfide
2,6-Dimethylaniline
Benzyl acetate
2,4-Dichlorophenol
1,2,4-Trichlorobenzene
p-Dibromobenzene
2,3-Dimethylphenol
m-Chloroaniline
α-Terpineol
Benzthiozole
o-Isopropylphenol
Quinoline
Tributylamine
Anisaldehyde
2-Nitrocresol
Dimethyladipate
Bis(2-chloroethoxy)ethane
3,4-Dichlorobenzaldehyde
Indole
n-Decanol
2-Methylnaphthalene
4-Chloro-3-methylphenol
p-*t*-Butylphenol
Phenyl carbamate
1,2,4,5-Tetrachlorobenzene
Tridecane
Nicotine
Biphenyl[b]
2,3,6-Trichlorophenol[b]
2,6-Dinitrotoluene
2,4-Dimethylquinoline
Tetradecane
Dimethyl phthalate
Methyl-*p*-toluene sulfonate
1,8-Dimethylnaphthalene
Acenaphthene
2,4-Dinitrotoluene
1-Naphthol
Di-*t*-butyl-4-methylphenol
Ethyl-*P*-toluene sulfonate
N,N-Dimethyldodecylamine
n-Pentadecane
2,4,5-Trimethylnaphthalene
1,2-Dichloronaphthalene
Fluorene
2-Aminobiphenyl
Diethylphthalate
Diphenylamine
4-Bromodiphenyl ether
n-Hexadecane
Tri-*n*-butyl phosphate
Hexachlorobenzene
Dibenzylamine
Atrazine
n-Heptadecane
Caffeine
Benzyl sulfide
Carbazole
n-Octadecane
1,8-Diaminonaphthalene
Diphenylsulfone
Diphenyl mercury
n-Nonadecane
Di-*n*-butyl phthalate
Aldrin
n-Eicosane
Pyrene

(continued)

Table 9.5 (cont'd.)

Compounds[a]

9,10-Dimethylanthracene
n-Heneicosane
Methyl stearate
n-Docosane
Butylbenzylphthalate
n-Tricosane
Triphenylphosphate
Chrysene
Di(ethylhexyl)phthalate
Tetraphenyl tin
Cholesterol

[a] Each compound is added to give approximately 100 μg/mL as the final concentration in methanol. Compounds are listed in elution order on a DB-1 fused silica capillary column.

[b] Coelute.

Super-Q or equivalent) may be used to generate reagent water.

(k) Alkane calibration standard - dissolve 50 mg each of *n*-decane, *n*-undecane, *n*-dodecane, *n*-tridecane, *n*-tetradecane, *n*-pentadecane, *n*-hexadecane, and *n*-heptadecane in 10 mL methylene chloride.

9.11 PREPARATION FOR ANALYSIS

9.11.1 Preparation of WABN System Performance Solution

(1) Prepare individual stock standards (12 mg/mL) of the compounds listed in Table 9.1.

- solids - accurately weigh 0.120 g of pure material, dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.
- liquids - with a 250 μL syringe accurately measure 120 μL of pure liquid; dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.

(2) Prepare secondary standard - with a 1 mL graduated pipette accurately measure 0.25 mL of each stock standard into a 10 mL volumetric flask [for quinoline and 6-*t*-butyl-*m*-cresol use 0.8 mL, for 4-fluoro-2-iodotoluene use 125 μL (measure with a 250 μL syringe), for 2,4,6-trimethylpyridine and *m*-cresol use 25 μL (measure with a 50 μL syringe), and for methyl stearate use 10 μL (measure with a 50 μL syringe)]. Dilute to volume using

methylene chloride to give the concentration in Table 9.1. Transfer into a Teflon sealed screw cap bottle. Store at 4°C. If degradation or evaporation has occurred, fresh standards should be prepared sooner.

(3) If compound purity is 96% or greater, the weight or volume can be used without correction to calculate the concentration of stock standards. If the compound is less than 96% pure, it cannot be used as a standard. Transfer the stock standards into Teflon sealed screw cap bottles for convenience in use, store at 4°C.

9.11.2 Preparation of Surrogate Standard Solution

(1) Prepare individual stock standards (12 mg/mL) of the compounds listed in Table 9.5.

- solids - accurately weigh 0.120 g of pure material, dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask.
- liquids - with a 250 µL syringe accurately measure 120 µL of pure liquid; dissolve in methylene chloride, dilute to volume in a 10 mL volumetric flask. If the density of a liquid is unknown, weigh an empty dry 250 µL syringe, fill with 120 µL of the pure liquid and reweigh.

(2) Prepare a secondary standard by accurately pipetting 0.40 mL of each stock standard into a 50 mL volumetric flask. Dilute to the mark with methylene chloride and mix well. Transfer into a Teflon sealed screw cap amber bottle and store at 4°C.

9.11.3 Cleaning of Materials

(1) All glassware should be washed with Amway S-A-8 laundry compound (or equivalent) rinsed with deionized water and heated for a minimum of 4 hours at 500 to 550°C in a glassware oven. All cleaned glassware is immediately capped or covered with foil to prevent contamination.

(2) Anhydrous sodium sulfate is cleaned and dried prior to use. Approximately 500 g of material is needed to process four samples and is the minimum amount of material which should be prepared. The following instructions describe procedures based on 500 g of

material; however, it is possible to prepare larger batches and store the prepared material in a sealed Erlenmeyer flask. If a larger quantity of material is prepared, volumes for rinsing should be adjusted accordingly.

Sodium sulfate (500 g) is placed in a 1 L Erlenmeyer flask with a ground glass joint. Methylene chloride (500 mL) is added, the flask is swirled for 5 minutes, and the solvent decanted. The procedure is repeated two additional times. To dry the sodium sulfate, the cleaned material is heated in the flask in a drying oven at 130°C overnight. The clean dried material can be stored in the drying oven, or stoppered and stored in a dessicator.

To prepare drying columns, a small piece of pre-extracted glass wool is placed in the bottom of the chromatography column, 13 cm of sodium sulfate (~85 g) is poured into the column, followed by an additional plug of pre-extracted glass wool. Columns should be used immediately or stored in a drying oven at 130°C. Rinse the Na_2SO_4 with 50 mL of extracting solvent just prior to using the column.

(3) Teflon liners and teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. The sonicated liners are vacuum-oven (~20 inches of water) dried for 3 to 5 hours at 70° and stored in clean, Teflon lined screw cap bottles.

(4) Glass wool is cleaned by placing it in the extraction chamber of a Soxhlet extractor and extracting overnight with pesticide grade methylene chloride. The glass wool is placed in a wide mouth jar and the solvent removed either under nitrogen stream or by warming. Cap and store until needed.

9.11.4 GC/FID Performance Evaluation

Prior to analyzing procedural blanks, acceptable performance for the GC/FID system must be demonstrated.

(1) Analyze the GC/MS system performance solution as specified in Table 9.2. Figure 9.3 shows a total ion chromatogram of this

mixture analyzed by GC/MS under similar conditions which may be used to identify test components in the standard.

(2) Measure peak asymmetry or tailing for acetophenone and 1-tetradecanol using the percent peak asymmetry factor (PAF):

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

where

B = the width from the back of the chromatographic peak to the perpendicular from the peak measured at 10% above baseline.

F = the width from the front of the chromatographic peak to the perendicular from the peak measured at 10% above baseline.

The PAF should measure less than 200% for both acetophenone and 1-tetradecanol.

(3) Check the acidity/basicity of the column by the peak area ratios determined by integrator or triangulation of 2,6-dimethylaniline and 2,6-dimethylphenol to acetophenone. A ratio of 0.7 to 1.3 for both is acceptable.

(4) Sensitivity is checked by measuring signal to noise ratios for 2,4,6-trimethylpyridine, m-cresol, and methyl stearate. A signal- to-noise ratio of 10 or greater for all three compounds is acceptable.

9.11.5 Procedural Blanks

If the BLLE procedure is also being performed with the same reagents, solvents and materials only Procedural Blank 2 need be prepared. Otherwise, for each lot of materials and reagents that are used, a set of three procedural blanks are required. Table 9.4 identifies these blanks and defines their purpose. These blanks must be processed and analyzed by GC/FID prior to processing any samples. If a large number of samples are to be processed, it is advantageous to prepare large lots of materials and reagents thereby reducing the number of blanks which must be run.

9.11.5.1 Procedural Blank 1--

(1) Prepare an extractor as described in step 9.12(4), then follow

the extraction procedure as described in steps 9.12(6) to 9.12(20), except step 9.12(13). Run extractor for only 60 min.

(2) Add 10 μL of the GC external standard to the blank concentrate. Agitate the K-D receiver to give a uniform distribution of standard in the concentrate.

(3) Analyze samples by GC/FID using the conditions described in Table 9.2. Contaminant peak heights should measure less than 20% relative to the standard.

(4) If the blank is acceptable, add an aliquot (10 μL) of the MS external standard solution. Agitate the K-D receiver to give a uniform distribution of standard in the concentrate. Transfer the concentrate to a 1 dram vial and cap. Store at 0°C until GC/MS analysis.

(5) If significant contamination is present, the extraction apparatus should be cleaned and the procedure repeated. If this blank is acceptable, then the extractor was the source of contamination.

(6) If significant contamination is present in this blank, fresh methylene chloride of a different lot or source should be used and the reagent blank repeated. When the reagent blank is acceptable, procedural blanks 2 and 3 may be processed.

9.11.5.2 Procedural Blanks 2 and 3--

(1) Prepare two extractors as described in step 9.12(4).4. For procedural blank 3, pour 1000 mL of reagent water into the extractor and proceed with the extraction [9.12(5) and following]. For procedural blank 2 use 1000 mL of reagent water spiked with 1 mL each of the sulfuric acid, sodium hydroxide, and sodium thiosulfate solutions to be used for sample collection (Chapter 4), and 10 mL of the phosphate buffer solutions [9.10(28)(i)].

(2) Follow the extraction procedure as described in steps 9.12(5) to 9.12(20).

(3) Add 10 μL of the GC external standard to the blank concentrates. Agitate the K-D receiver to give a uniform distribution of standard in the concentrates.

(4) Analyze the samples by GC/FID using the conditions in Table 9.2. Peak heights for contaminants in Procedural blank 3 which are

not present in procedural blank 2 should measure less than 20% relative to the external standard.

(5) If significant contamination is present in Procedural blank 3 which is not present in procedural blank 2, the procedure should be repeated using fresh solutions of sulfuric acid, sodium hydroxide, sodium thiosulfate, and phosphate buffer until an acceptable blank is achieved.

9.12 SAMPLE EXTRACTION

(1) Mark the interface between the keeper solvent and aqueous layer on the sample bottle with an indelible mark.

(2) Transfer the keeper solvent to a clean 1 L Erlenmeyer flask using a transfer pipet.

(3) Adjust the pH to 8.0 using 6N NaOH or 1:1 H_2SO_4. Check the pH with narrow range pH paper. Add 10 mL of 1.0 M pH 8.0 phosphate buffer.

(4) With the extractor stopcock closed, pour 275 mL of methylene chloride into the extractor body.

(5) Slowly add the sample without disturbing the organic layer. Use a glass funnel so that the sample slowly runs down the sides of the extractor. Rinse the sample bottle with ~10 mL of methylene chloride and transfer to the extractor with the funnel tip extending into the methylene chloride in the extractor.

(6) Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

(7) Mount the motor and stirring rod above the extractor so that the paddles on the stirring rod are just below the aqueous surface (mark positions for reference) (Figure 9.8).

(8) With the stopcock closed, add 225 mL of methylene chloride to the distillation flask along with cleaned boiling chips.

(9) Position the condenser, heating mantle, and variable transformer (Figure 9.8).

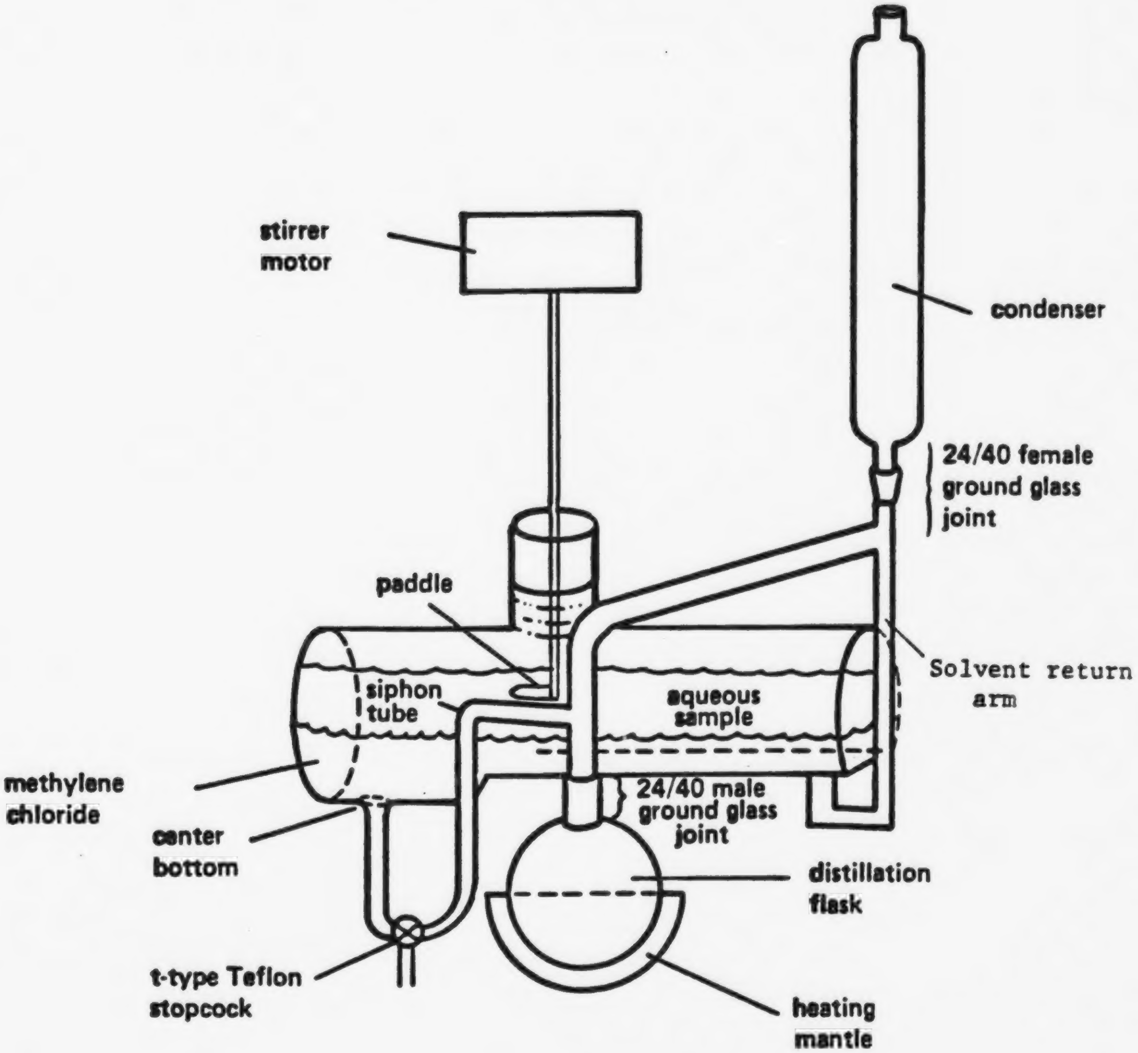

stirrer motor
condenser
24/40 female ground glass joint
paddle
siphon tube
aqueous sample
Solvent return arm
methylene chloride
24/40 male ground glass joint
center bottom
distillation flask
t-type Teflon stopcock
heating mantle

Figure 9.8. Flow-under extractor with attachments.

(10) Adjust the variable transformer to give a 20 mL/min distillation rate (the variable transformer should be calibrated before an actual sample is extracted).

(11) Open the stopcock to allow solvent to cycle through the siphon tube when the methylene chloride in the flask starts to distill over.

(12) Turn on the stirring motor at a speed fast enough to suspend any solids present but slow enough not to break the sample-solvent interface.

(13) Extract sample for 3.5 hr.

(14) Close stopcock, turn off stirrer, and remove heating mantle.

(15) Remove solvent from distillation flask and transfer to the 1 L Erlenmeyer flask containing the keeper solvent.

(16) Rinse the distillation flask three times with 5 mL portions of methylene chloride and add rinse to the same Erlenmeyer flask.

(17) Remove water from the methylene chloride by adding ~5 g of cleaned and dried sodium sulfate. Swirl the flask for several minutes. Pour the semi-dried extract through a sodium sulfate drying column using a glass funnel to prevent spillage. Rinse bulk sodium sulfate and Erlenmeyer flask and drying column with an additional 5 mL of solvent and add rinsings to eluate.

(18) The eluate and rinse solvent are collected in a 500 mL K-D flask equipped with 4 mL concentrator tube containing several boiling chips. Pre-calibrate the concentrator tube to compensate for the volume of the boiling chips. Attach the three-ball macro-Synder column. Place the K-D apparatus on a warm water bath with the concentrator tube partially immersed in water or on a steam bath. Adjust the temperature of the water bath such that evaporating solvent causes the balls in the Synder column to chatter actively but does not flood the chambers of the column. Under these conditions evaporation of 500 mL of methylene chloride to 2 mL should take 20 to 30 minutes.

(19) When the liquid has reached an apparent volume of 2 mL remove the K-D apparatus from the heat and allow to cool. Remove the Snyder column. With 1 to 2 mL of solvent, rinse the evaporative

flask and its lower joint into the concentrator tube. The final volume in the concentrator tube should be approximately 4 mL.

(20) Attach a modified micro-Snyder column to the K-D receiver. Connect a transfer pipette by Teflon tubing to the nitrogen manifold. Place the pipette in the modified Synder column above the solvent level (Figure 9.4). Gently blow a stream of nitrogen above the solvent surface until the volume is reduced to approximately 1 mL. Rinse the sides of the concentrator tube with approximately 0.5 mL of solvent. Reduce volume of extract to 1.0 mL. Record final volume.

(21) To determine whether the extract is so complex that it must be cleaned up, a 1 µL injection is made on a gas chromatograph with FID detection. The 180 cm × 2 mm i.d. column is packed with 3% OV-1 on Chromosorb W HP. A temperature program from 30°C to 250°C at 10°/min is used. The attenuation is set to give 30 to 70% of full scale deflection for the various peaks of the alkane calibration standard [9.10(28)(k)]. The area produced by all eight alkanes is Area C. Areas may be measured by planimeter.

(22) The extract chromatogram from step 21 is evaluated as follows: The baseline rise (A) is estimated by connecting the valleys of the chromatogram as shown in Figure 9.6. Draw a zero line (B) horizontal to the level of the baseline before injection. At any point after the first 2 minutes of the chromatogram where line A is more than 5% of full scale above line B begin an incremental integration of the area between A and B. This is repeated in 5% increments as indicated by the shaded areas in Figure 9.6. Compute the area between A and B which is shaded (Area A-B). Measure the area of the peaks produced by the alkane calibration standard (Area C) and compute the mg/mL equivalent of the baseline rise M_A by

$$M_A = \frac{\text{Area}_{A-B} \times 40 \text{ mg/mL}}{\text{Area}_C}$$

A sample must be fractionated if $M_A > 50$ mg/mL (go to Section D). Otherwise it may be analyzed by GC/MS without further workup.

(23) If no fractionation is required add 25 μL of external standard solution to the sample concentrate. Agitate the K-D receiver to give a uniform distribution of external standard in the sample concentrate. Transfer a measured portion of the sample concentrate to a 1 dram vial and cap. Store at 0°C until analysis by GC/MS.

(24) Proceed to Section E -GC/MS/COMP ANALYSIS OF WABN-SC, WABN-BL, OR WABN-FU SAMPLE EXTRACTS.

D. CLEANUP/FRACTIONATION OF WABN FRACTIONS

9.13 INTRODUCTION

This protocol describes a procedure for fractionating WABN extracts identified by the scouting test [9.8(15-16) and 9.12(21-22)] as containing high levels of chromatographable organics. These high levels of organic substances would prevent the identification and quantitation of analytes at the levels specified for the overall methods.

The fractionation is performed using a 5 g silica gel open column. The silica gel is activated at 150°C and deactivated with 14 mL of water per 100 g of silica gel. The sample is then fractionated into three fractions consisting of pentane (20 mL), 20% methylene chloride in pentane (50 mL) and 6% methanol in methylene chloride (100 mL). The second two fractions may be recombined for GC/MS/DS analysis.

The mean percent recoveries and coefficients of variation for this procedure alone are given in Table 9.6.

9.14 APPARATUS AND REAGENTS

The following materials are required for processing a set of nine sample extracts. The fractionation of nine sample extracts will require one and a half working days including solvent evaporations.

(1) Nine chromatography columns 220 mm × 10 mm i.d. with Teflon stopcocks and 24/40 ground glass joints at the top (Figure 9.9).

(2) Nine 250 mL dropping funnels with 24/40 ground glass joints and Teflon stopcocks.

(3) Nine glass funnels to fit dropping funnels.

(4) Pre-extracted glass wool (~50 mL) for columns. Glass wool should be precleaned by Soxhlet extracting overnight with methylene chloride.

(5) One Soxhlet extractor (50 mL capacity minimum) for extracting glass wool.

(6) Nine Kuderna-Danish apparatus. Each apparatus consists of

   (a) Three-ball macro-Snyder columns (Kontes #K503000) - 300 mm length with 24/40 joints,

   (b) Evaporative flask (Kontes #K570001) - 250 mL with 24/40 top joint and 19/22 lower joint,

Table 9.6. RECOVERIES[a] AND ELUTION PROFILES FOR CLEANUP/FRACTIONATION BY SILICA GEL OPEN-COLUMN[b]

Compound	Fraction 1[c]	Fraction 2[d]
Pyridine[e]		
α-Picoline		75 ± 13
2-Heptanone		61 ± 30
o-Xylene	82 ± 5.6	
t-Butyl carbamate[e]		
2-*n*-Butoxyethanol		108 ± 15
t-Butyl propionate		51 ± 14
Benzaldehyde		84 ± 23
Aniline		75 ± 8.5
Lutidene[e]		
2-Octanone		78 ± 27
Phenol		61 ± 18
Benzyl chloride	69 ± 5.5	trace
2,3,6-Trimethyl pyridine		80 ± 19
Decane		84 ± 3.2
Salicylaldehyde[f]		
Acetophenone		88 ± 6.6
Phenyl acetate		62 ± 19
Phenyl ethanol		87 ± 7.9
Nitrobenzene		67 ± 22
Tolualdehyde		64 ± 16
2,3-Dihydrobenzofuran		44 ± 5.2
p-Cresol		86 ± 7.4
Fenchone		85 ± 10
3-Chlorobenzaldehyde		99 ± 17
Isophorone		87 ± 13
2-Bromo-1-chlorobenzene	76 ± 5.5	
o-Chloroanisole		41 ± 8.0
4-Chlorobenzonitrile		46 ± 17
1,4-Dicyanobutane		53 ± 18
Di-*t*-butyldisulfide	77 ± 4.5	
2,6-Dimethylaniline		88 ± 7.0
Benzyl acetate		77 ± 10
2,4-Dichlorophenol		64 ± 14
1,2,4-Trichlorobenzene	76 ± 5.1	
Naphthalene	79 ± 4.9	
p-Dibromobenzene	87 ± 5.4	
2,3-Dimethylphenol		85 ± 9.5
m-Chloroaniline		150 ± 5.1
α-Terpineol		81 ± 11
Benzthiozole		87 ± 9.6
o-Isopropylphenol		77 ± 5.6
Quinoline		69 ± 14
Tributylamine		54 ± 6
Anisaldehyde		86 ± 13

(continued)

Table 9.6 (cont'd.)

Compound	Fraction 1[c]	Fraction 2+3[d]
2-Nitro-m-cresol		49 ± 22
Dimethyladipate		79 ± 11
Bis(2-chloroethoxy)ethane		84 ± 4.0
3,4-Dichlorobenzaldehyde		54 ± 14
Indole		72 ± 10
Decanol		86 ± 6.7
2-Methylnaphthalene	67 ± 9.0	trace
4-Chloro-3-methyl phenol		87 ± 14
p-t-Butylphenol		95 ± 9.5
Phenyl carbamate		120 ± 36
1,2,4,5-Tetrachlorobenzene	77 ± 9.0	
Tridecane	87 ± 6.2	
Nicotine[g]		
2,3,6-Trichlorophenol	46 ± 12	35 ± 3.7
2,6-Dinitrotoluene		80 ± 9.0
2,4-Dimethylquinoline		81 ± 7.9
Tetradecane	93 ± 5.9	
Dimethyl phthalate		93 ± 10
Methyl-p-toluene sulfonate[e]		
1,8-Dimethylnaphthalene	68 ± 3.1	
Acenaphthene	63 ± 7.1	22 ± 14
2,4-Dinitrotoluene		77 ± 12
1-Naphthol		83 ± 16
Di-t-butyl-4-methyl phenol	71 ± 5.2	12 ± 8.3
Ethyl-p-toluene sulfonate		110 ± 19
N,N-Dimethyldodecyl amine		75 ± 12
Pentadecane	91 ± 4.5	
2,3,5-Trimethylnaphthalene	57 ± 11.2	31 ± 15
1,2-Dichloronaphthalene	88 ± 7.3	
Fluorene	27 ± 21	58 ± 7.9
2-Aminobiphenyl		82 ± 1.5
Diethyl phthalate		100 ± 10
Diphenyl amine		78 ± 6.7
(4-Bromodiphenyl) ether	31 ± 21	55 ± 8.0
Hexadecane	99 ± 3.3	
Tri-n-butyl phosphate		110 ± 10
Hexachlorobenzene	95 ± 5.9	
Dibenzylamine		70 ± 14
Atrazine		87 ± 16
Heptadecane	99 ± 5.5	
Acridine		92 ± 6.0
Anthracene	50 ± 9.0	48 ± 8.8
Caffeine		74 ± 18
Benzyl sulfide		82 ± 11
Carbazole		100 ± 15

(continued)

Table 9.6 (cont'd.)

Compound	Fraction 1[c]	Fraction 2+3[d]
Octadecane	98 ± 6.7	
1,8-Diaminonaphthalene		30 ± 15
Diphenyl sulfone		71 ± 9.0
Diphenyl mercury		82 ± 8.8
Nonadecane	103 ± 3.6	
Di-*n*-butyl phthalate		110 ± 12
Aldrin	96 ± 2.9	
Eicosane	110 ± 6.9	
Pyrene	34 ± 9.1	62 ± 17
9,10-Dimethyl anthracene	17 ± 25	85 ± 1.0
Heneicosane	99 ± 11	
Methyl stearate		70 ± 6
Docosane	92 ± 9.3	
Butylbenzyl phthalate		79 ± 12
Tricosane	89 ± 8.3	
Triphenyl phosphate		86 ± 5.6
Chrysene		71 ± 5.0
Di(ethyl hexyl) phthalate		104 ± 18
Tetraphenyl tin	trace	54 ± 20
Perylene		98 ± 21
Cholesterol		71 ± 27

[a] Mean ± coefficient of variation (n=3), nominal spiking level was 10 ppb.

[b] 5 g of silica gel (100/120 mesh) activated overnight at 150°C, deactivated by 14 mL deionized water/100 g silica gel.

[c] Extract solution (10 mL vol. ~1% methylene chloride in pentane) + 10 additional mL pentane.

[d] 50 mL 20% methylene chloride in pentane and 100 mL 6% methanol in methylene chloride.

[e] Did not chromatograph under conditions used.

[f] Interference prevented quantitation.

[g] Not recovered.

(c) Concentrator tube (Kontes #K570000) - 4 mL graduated, with 19/22 joint. Attach to evaporative flask with springs (Kontes #K50300-0232). Ground glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

(7) Eighteen modified micro-Snyder columns (Kontes #K569251) with 19/22 joint.

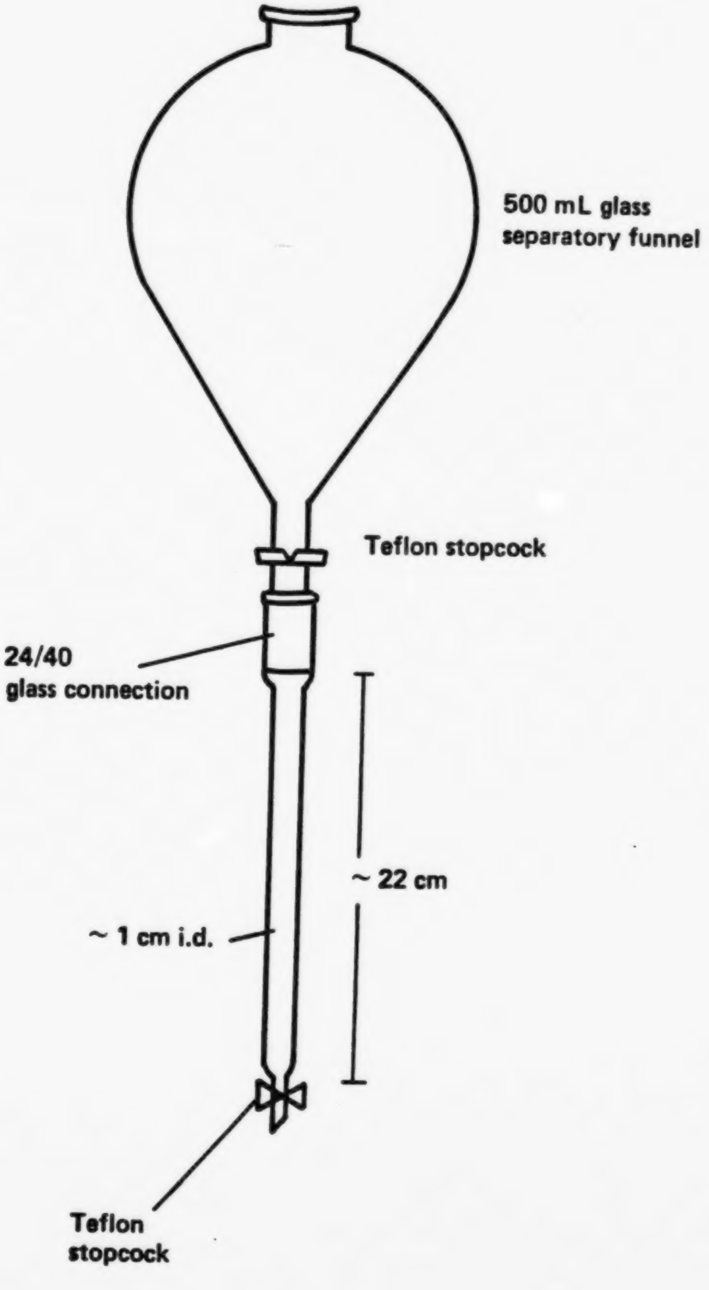

500 mL glass
separatory funnel
Teflon stopcock
24/40
glass connection
~ 22 cm
~ 1 cm i.d.
Teflon
stopcock

Figure 9.9. Chromatography column with sample reservoir (exact dimensions of column are not critical).

(8) Eighteen concentrator tubes (Kontes #K570050-25) - 25 mL graduated, with 19/22 joint.

(9) Boiling chips - Hengar granules are ground with a mortar and pestle and sieved to obtain a 60/80 mesh. These granules are cleaned by extracting ~5 g three times with ~150 mL methylene chloride.

(10) Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

(11) One manifold with a temperature controlled water bath for nitrogen blowdown. A manifold with nine spaces is preferred but not essential.

(12) Nine glass vials (1 dram) with 13 mm screw caps (Supelco 2-3213) and Teflon lined rubber septa (Supelco 2-3216).

(13) One gas chromatography column (180 cm × 2 mm i.d.) packed with 3% OV-1 on Chromosorb W HP (80/100 mesh) or equivalent.

(14) One gas chromatograph with flame ionization detector.

(15) One laboratory oven.

(16) Materials and Reagents

  (a) 1.5 L methylene chloride (Burdick and Jackson, distilled in glass).

  (b) 0.5 L pentane (Burdick and Jackson, distilled in glass).

  (c) 50 mL methanol (Burdick and Jackson, distilled in glass).

  (d) 100 g silica gel 100/120 mesh (Fisher reagent grade).

  (e) 1 mL column performance standard (Table 9.7).

  (f) Reagent water - reagent water is defined as a water source which does not produce a background interference at the

Table 9.7. COLUMN PERFORMANCE STANDARD

Compound	Concentration mg/10 mL[a]
Naphthalene	10
Anthracene	20

[a] Methylene chloride solvent.

limit of detection. A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

9.15 PREPARATION FOR FRACTIONATION

(1) Cleaning of Glassware

All glassware to be used should be washed with Amway S-8-A, rinsed with distilled deionized water and heated to 500 to 550°C in a glassware oven for a minimum of 4 hours. Cleaned glassware is immediately capped or covered with foil to prevent contamination.

(2) Activation of the Silica Gel

Silica gel (100 g) is placed in a wide mouth jar in an oven at 105°C for a minimum of 14 hr. Immediately prior to use cap the jar and place in a desiccator to cool. When cool add 14 mL of reagent water and agitate until evenly distributed. Allow to equilibrate for at least 2 hrs.

(3) Cleaning Glass Wool

Glass wool is cleaned by placing in the extraction chamber of a Soxhlet extractor and extracting overnight with pesticide grade methylene chloride. The glass wool is placed in a wide mouth jar and the solvent removed either under nitrogen stream or by warming. Cap and store until needed.

9.16 SAMPLE FRACTIONATION

9.16.1 Determination of Silica Gel Activity

(1) A chromatography column containing 5.00 ± 0.05 g of silica gel is prepared by layering the silica gel on top of a plug of glass wool and tapping the column gently to settle the sorbent.

(2) Dilute 0.5 mL of the column performance standard (Table 9.7) to 10 mL with pentane and mix gently.

(3) Transfer the diluted column performance mixture to the silica gel column and allow the solution to elute through the column until the solvent reaches the top of the silica gel. Collect this eluate.

(4) Elute the column with an additional 10 mL of pentane and collect this eluate as above (3) in the same container. This should be ~15 mL.

(5) Dilute 0.5 mL of column performance standard with 15 mL of pentane and mix.

(6) Inject the column eluate (4) on the 180 cm × 2 mm i.d. 3% OV-1 on Chromosorb W HP packed GC column. Compare peak areas (or heights) directly against those obtained from an equal volume injection of the diluted performance standard (5).

(7) If the naphthalene recovery is <70% and no anthracene is observed, reduce the amount of silica gel in the column (1) by 0.50 g and repeat steps 1-6. If the naphthalene recovery is >70% and the anthracene recovery is ≥50%, increase the amount of silica gel by 0.50 g and repeat steps 1-6. If the naphthalene recovery is >70% and the anthracene recovery is <50% proceed with samples.

9.16.2 Fractionation of Samples

(1) Adjust extract volumes to 1.0 ± 0.2 mL with methylene chloride.

(2) Transfer the sample extracts with 9 mL of pentane to a 25 mL K-D concentrator tube and evaporate to 1.0 mL under a nitrogen stream with a modified micro-Snyder column. Connect a transfer pipette by Teflon tubing to a nitrogen manifold. Place the transfer pipette in the modified Snyder column above the liquid level (Figure 9.4). Gently blow a stream of nitrogen above the liquid surface until the volume is reduced to approximately 1 mL. Rinse the sides of the concentrator tube with approximately 0.5 mL of solvent.

(3) Redilute the extracts with 9.0 mL of pentane.

(4) Prepare nine columns using the amount of silica gel determined in Section 9.16.1. The silica gel is layered over a glass wool plug and the column tapped to settle the sorbent.

(5) The rediluted extract is transferred to a column and the solvent eluted until it reaches the surface of the silica gel. Collect this eluate in a 25 mL K-D concentrator tube.

(6) Rinse the extract container in 2 mL portions with a total of 10.0 mL of pentane transferring each portion to the column. Elute this solvent until the liquid level reaches the sorbent and close the stopcock. Collect this eluate with that from

step 5 and label as Fraction I. The volume of eluate should be ~15 mL.

(7) Transfer the eluate to a 250 mL K-D evaporator flask fitted with a 4 mL graduated concentrator tube.

(8) Measure 50 mL of 20% methylene chloride, 80% pentane (v/v). Rinse the sample extract container (from step 2) three times with 2 mL portions of this solvent and transfer to the column. Add remaining solvent to column using a droping funnel (Figure 9.9). Elute the column until the solvent reaches the top of the silica gel. Collect in a 250 mL K-D flask equipped with 4 mL concentrator tube containing several boiling chips. The concentrator tube should be precalibrated to compensate for the volume of the boiling chips.

(9) Measure 100 mL of 6% methanol in methylene chloride. Rinse the sample extract container three times with 2 mL portions of this solvent and transfer to the column. Add remaining solvent to the column using a dropping funnel. Elute the column until the solvent reaches the top of the silica gel. Label the combined eluates from steps 8 and 9 as Fraction II.

(10) Evaporate Fraction I to 1.0 mL by nitrogen blowdown with a modified micro-Snyder column as described in step 1.

(11) Evaporate the eluate labeled Fraction II. Attach the three-ball macro-Snyder column. Place the K-D apparatus on a warm water bath with the concentrator tube partially immersed in water or on a steam bath. Adjust the temperature of the water bath such that evaporating solvent causes the balls in the Snyder column to chatter actively but does not flood the chambers of the column. Under these conditions evaporation of 150 mL of solvent to 2 mL should take 10 to 15 minutes.

(12) When the liquid has reached an apparent volume of 2 mL remove the K-D apparatus from the heat and allow to cool. Remove the Snyder column. With 1 to 2 mL of solvent, rinse the evaporation flask and its lower joint into the concentrator tube. The final volume in the concentrator tube should be approximately 4 mL.

(13) Attach a modified micro-Snyder column to the concentrator tube and concentrate to 1.0 mL as described in step 2.

(14) Add 25 µL of MS external standard solution [Part III, Section 9.10(28)(f)] to each sample concentrate. Agitate the K-D receiver to give an uniform distribution of external standard in the sample concentrate. Transfer a measured portion of the sample concentrate to a one dram vial and cap. Store at 0°C until GC/MS analysis.

(15) Proceed to Section E - GC/MS/COMP ANALYSIS OF WABN-SC, WABN-BL, OR WABN-FU SAMPLE EXTRACTS.

E. GC/MS/COMP ANALYSIS OF WABN-SC, WABN-BL, OR WABN-FU SAMPLE EXTRACTS

9.17 INTRODUCTION

Sample extracts containing weak acids, weak bases, and neutrals (pH 8.0 extractables) obtained from protocols I, II, III or IV are analyzed using the conditions prescribed here. Prior to beginning GC/MS/COMP analysis of these fractions the analyst should be familiar with the procedures described in Chapter 13.

9.18 PREPARATION FOR ANALYSIS

9.18.1 GC/MS/COMP Operating Parameters

The recommended GC/MS operating parameters are given in Table 9.10. It is also recommended that the capillary used here not be used for the analysis of fractions from other protocols.

9.18.2 MS Calibration

The mass spectrometer is calibrated using the manufacturer's recommended approach (Chapter 13). The acceptability of the calibration results is verified with the analysis of the system performance solution (SPS).

9.19 GC/MS/COMP ANALYSIS

9.19.1 SPS (Quality Control)

Using the prescribed GC/MS conditions the SPS (Table 9.1) is analyzed and all necessary data is acquired and evaluated prior to proceeding to sample analysis. The SPS is analyzed at the beginning of each day of operation.

The SPS raw data is extracted from the run, and test parameters calculated and plotted or tabulated (Tables 9.11 and 9.12). Up to 14 days of SPS analytical results can be historically recorded in Table 9.11 for day-to-day comparisons. This allows the analyst to anticipate GC/MS maintenance requirements. Calculations of test parameters and discussion of their use are given in Chapter 13.

A check of RMRs on a daily basis is also calculated from SPS raw data and tabulated (Table 9.12). The RMRs are compared to those obtained during the development of the historical data bank. The usage of this data is discussed in Chapter 13.

Table 9.10. GC/MS OPERATING CONDITIONS
FOR EXTRACTABLE WEAK ACIDS, BASES AND NEUTRALS

GC Column	30 m DB-1 (1.0 μ film thickness) fused silica wide bore (0.34 mm I.D.) capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through column; 9:1 split injection
Carrier Gas Sweep Time	85 sec (50°C)
Temperature Program	50°C/5 min; to 250°C @ 4°/min and hold
Injector Temperature	250°C
Transfer Line Temperature	255°C
Injection Mode	Splitless, 0.4 min/split
Injection Volume	1.0 μL
Ionizing Energy	70 eV
Ion Source Temperature	250°C
Scan Range	35-600
Scan Speed	Scan 0.95 sec, hold 0.05 sec

9.19.2 Determination of RMRs

RMRs for the User's data bank (as opposed to the daily RMR checks discussed above) are determined by injecting a solution containing the internal standards and analytes.

The GC/MS/COMP operating parameters are given in Table 9.10. Table 13.8 may be used to tabulate RMR raw data.

9.19.3 Quality Control and Field Samples

After all specified criteria for GC and MS performance (Table 9.11) have been met, analysis of quality control or sample extracts is conducted.

A set of sample extracts may have been derived from laboratory and field blanks, surrogates, and collected field samples, and are generally analyzed in that respective order.

Qualitative and quantitative procedures are described in Chapter 13. Table 9.13 may be used to tabulate sample analyte raw data prior to using the MASQUANT software program or manual interpretation.

Table 9.11. GC-MS SYSTEM PERFORMANCE TEST FOR WEAK ACIDS, BASES AND NEUTRALS (WABN, pH 8 EXTRACTABLES)

Dates:

Run Id Code

GC Column and Program:

Notebook References:

MS SYSTEM CHECKS

High/Low Mass Balance Ion	DFTPP Relative Abundance Criteria
51	30% to 60% of m/z 198
68	<2% of m/z 69
70	<2% of m/z 69
127	40% to 60% of m/z 198
197	<1% of m/z 198
198	100%
199	5-9% of m/z 198
275	10% to 30% of m/z 198
365	at least 1% of m/z 198
441	present, but < m/z 443
442	>40% of m/z 198
443	17% to 23% of m/z 442

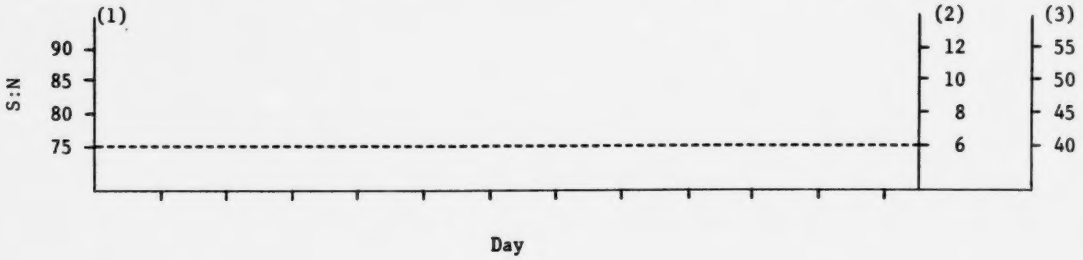
m/z	51	68	70	127	197	198	199	275	365	331	442	443	Pass/ Fail	Remedial Action
Abundance * Criteria	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )		
Day														

*These abundances are initially established by the MAS user, and subsequently become guidelines for acceptability of tune.

(continued)

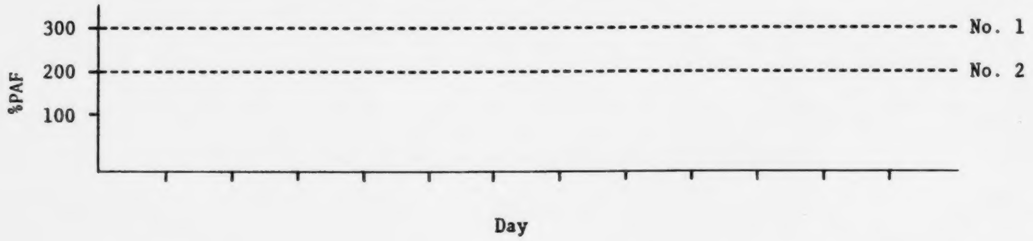
Table 9.11 (cont'd.)

Test	Components	Criteria
Limit of Detection	1. 2,4,6-Trimethylpyridine ● 2. m-Cresol x 3. Methyl Stearate ■	S:N (m/z 122) > 75:1 S:N (m/z 109) > 6:1 S:N (m/z 74) > 40:1

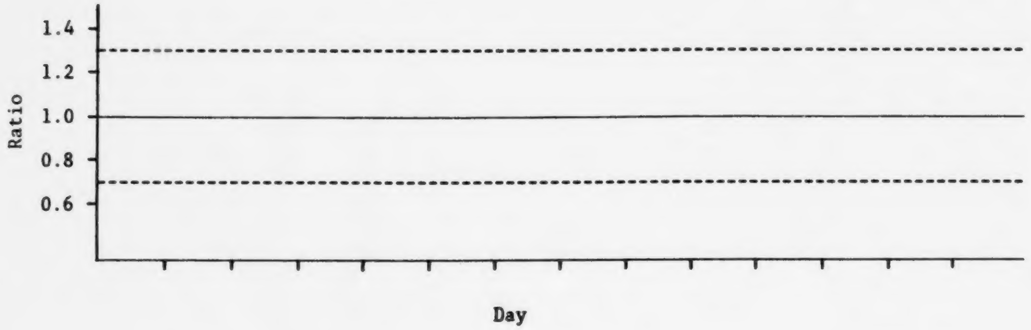


GC SYSTEM CHECKS

Peak Asymmetry	1. Acetophenone (TIC) ● 2. 1-Tetradecanol (TIC) ■	%PAF <300 %PAF <200



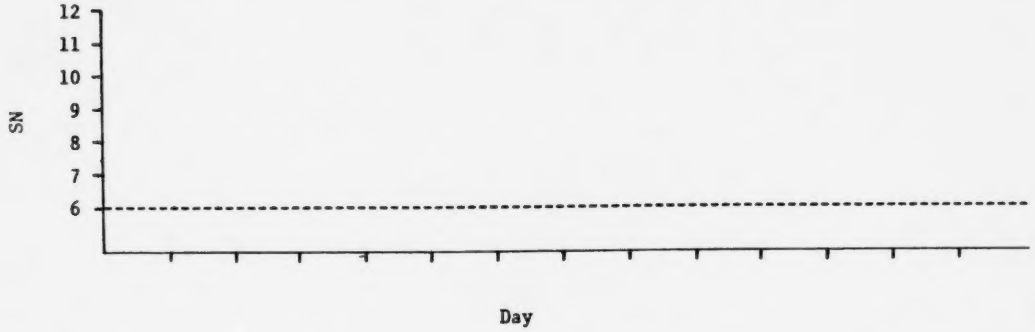
Acidity/ Basicity	1. Acetophenone (TIC) 2. 2,6-Dimethylphenol (TIC) ● 3. 2,6-Dimethylaniline (TIC) ■	Ratio 2:1 = 0.7 to 1.3 Ratio 3:1 = 0.7 to 1.3



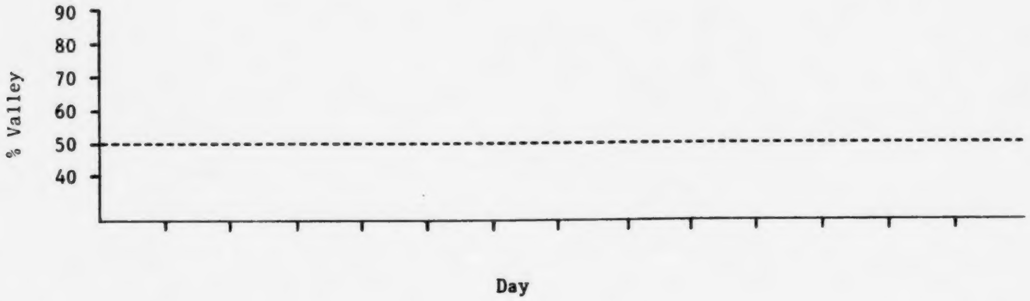
(continued)

Table 9.11 (cont'd.)

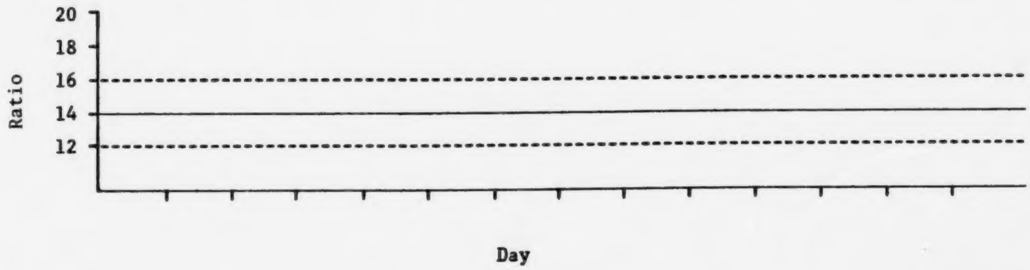
Test	Components	Criteria
Separation Number	n-Eicosane (m/z 43) n-Heneicosane (m/z 43)	SN ≥ 6



Test	Components	Criteria
Resolution	n-Octadecane (TIC) 1-Octadecene (TIC)	R = minimum 50% Valley



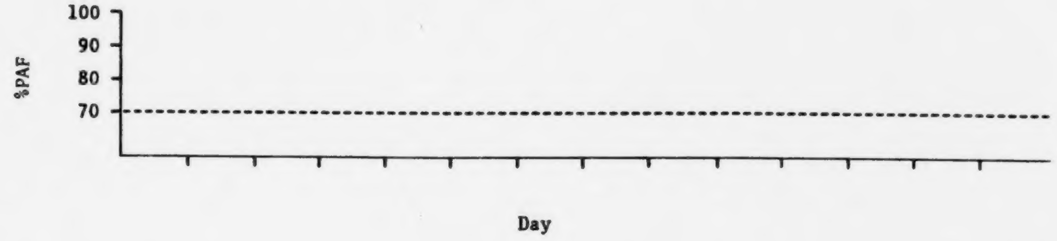
Test	Components	Criteria
Inertness	Methyl Stearate	Ratio m/z 74 to 298 ≤ 14:1



(continued)

Table 9.11 (cont'd.)

Test	Components	Criteria
Capillary Capacity	1. 6-*t*-butyl-*m*-cresol (TIC) ● 2. Quinoline (TIC) ■	%PAF >70



Relative Retention Shift	1. Heneicosane 2. Pyrene	RRS = 0.95-1.05

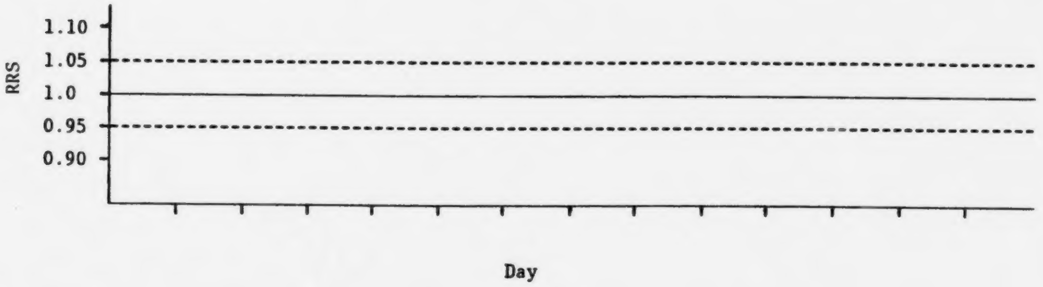


Table 9.12. GC-MS SYSTEM PERFORMANCE TEST: RMR CHECK FOR WABN EXTRACTABLES (pH 8.0)

Date: ____________________ **Run ID Code:** ____________________

DATA

Standard	MW	Amount (µg)	µM	Ion (m/z)	Area (Run 1)	Area (Run 2)	Area (Run 3)
4-Fluoro-2-Iodotoluene (FIT)	236			109			
				236			
d_{10}-o-xylene	116			98			
				116			
d_8-naphthalene	136			136			
d_5-nitrobenzene	128			82			
				128			
d_5-phenylethanol	112			84			
				112			
d_5-propiophenone	110			82			
				110			
d_5-acetophenone	125			110			
				125			
d_{12}-perylene	264			264			
d_9-acridine	188			188			
d_5-phenol	99			99			

MATRIX OF STANDARD ION RMRs

Standard	Ion	109	236	98	116	136	82	128	84	112	82	110	110	125	264	188	99
FIT	109																
	236																
d_{10}-o-xylene	98																
	116																
d_8-naphthalene	136																
d_5-nitrobenzene	82																
	128																
d_5-phenylethanol	84																
	112																
d_5-propiophenone	82																
	110																
d_5-acetophenone	110																
	125																
d_{12}-perylene	264																
d_9-acridine	188																
d_5-phenol	99																

COMMENTS:

Table 9.13. RAW DATA FOR WABN (-SC,-BL, OR-FU) FRACTION

Date: ____________

Run I.D. Code: ____________

Sample Identification: ________

Notebook Reference: ________

Vol. Water (L): ____________
(to which iS were added)

Vol. Water Processed (mL): ____

STANDARDS

	I.D. No.	Spectrum No.	Weight (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
4-Fluoro-2-Iodo-toluene				109,236:	,		,
d_{10}-o-Xylene				98,116:	,		,
d_8-Naphthalene				136:	,		,
d_5-Nitrobenzene				82,128:	,		,
d_5-Phenylethanol				84,112:	,		,
d_5-Propiophenone				82,110:	,		,
d_5-Acetophenone				110,125:	,		,
d_{12}-Perylene				264:	,		,
d_9-Acridine				188:	,		,
d_5-Phenol				99:	,		,

ANALYTES

Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Area	MW_x^a
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	

---

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{RMR_{x/y}} \times \frac{ng_y}{A_y \cdot MW_y}$$

$$K = \frac{ng_y}{A_y \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

CHAPTER 10

ANION-EXCHANGE, DISTILLATION AND ANALYSIS OF VOLATILE STRONG ACIDS (VOSA)

10.1 INTRODUCTION

10.1.1 Principle of the Technique

This technique describes the determination of volatile carboxylic acids (C_3 to C_8 acids) in drinking water, surface waters, and treated municipal and industrial wastewater effluents. Low molecular weight carboxylic acids are polar, water soluble compounds which will not easily extract into organic solvents. In addition, these acids in their protonated form are sufficiently volatile to give significant losses during solvent evaporation procedures. The procedure utilizes a strong anion exchange resin to concentrate these acids from the aqueous sample. The acids are eluted from the resin using sodium bisulfate in an acetone:water solution. Bisulfate both protonates acids to their nonionic forms which are then rinsed off the resin with acetone:water, and displaces acid salts from the resin material. Volatile acids are separated from the sodium bisulfate and nonvolatile sample components by distillation. Carboxylic acids are derivatized prior to GC/MS analysis to prevent adsorptive losses during chromatography. Benzyl bromide was selected as the derivatizing reagent for several reasons: carboxylic acids are derivatized quantitatively; the derivatizing reagent adds a large nonvolatile group to the acids to separate derivative from the solvent front; and derivatives are easily detectable during GC/MS analysis.

10.1.2 Detection Limits

If an average detection limit for GC/MS analysis for derivatized carboxylic acids is estimated as 10 ng per component, and the final volume of derivatized sample is 0.3 mL, then detection limits for a 1 μL injection are directly related to sample size.

1 ppb for a 2.5 L sample (drinking water);

5 ppb for a 500 mL sample (surface water);

10 ppb for a 250 mL sample (industrial and municipal wastewater effluents); and

50 ppb for a 50 mL sample (e.g., complex energy effluents).

10.1.3 Interferences

Background from the ion exchange resin can cause interferences for the analysis of acetic and propanoic acids. The measured levels of acetic and propanoic acids found in procedural blanks must be subtracted from the amount found in water samples to determine actual concentrations. Chloride ion on the ion exchange resin will interfere with eluant concentration and derivatization. Methods for removing chloride ion from the resin material are given.

10.1.4 Precision, Accuracy, and Scope

Table 1.7 in Chapter 1 presents average recoveries and standard deviations for the range of carboxylic acids determined in drinking water and municipal/industrial wastewater. This method gives acceptable recoveries for short chain carboxylic acids (C_3-C_8)

10.2 APPARATUS AND REAGENTS

The following materials are required for processing a set of nine samples plus three procedural blanks. Nine is the maximum number of samples which should be processed at a time, and includes certain quality control samples as listed in Table 5.1, Chapter 5. The three procedural blanks should be run before any samples are collected or processed. It will require two working days to process nine quality control and field samples.

(1) Nine glass chromatography columns, ~1 cm I.D. × 22 cm equipped with 500 mL sample reservoir (Figure 10.1). The size of the sample reservoir is not critical; however a 500 mL reservoir will hold the entire sample for all water types except drinking water. For drinking water an adaptor (Figure 10.2) for siphoning the sample onto the resin column should be used *in lieu* of a sample reservoir. This allows unattended flow through the sample for a 14 to 16 hr period such that the sample can be concentrated overnight. A vacuum aspirator is needed to start sample flow through the siphoning adaptor and resin column.

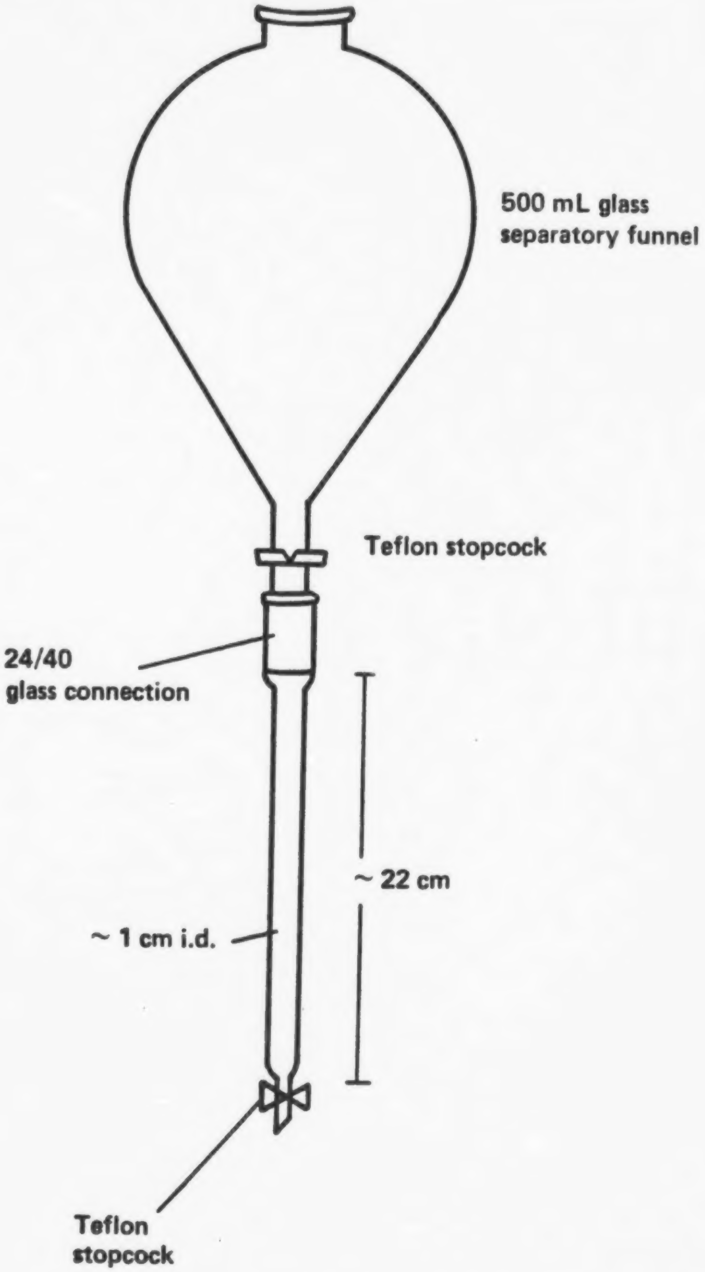

500 mL glass separatory funnel
Teflon stopcock
24/40 glass connection
~ 22 cm
~ 1 cm i.d.
Teflon stopcock

Figure 10.1. Chromatography column with sample reservoir (exact dimensions of column are not critical - must hold 10 mL of resin).

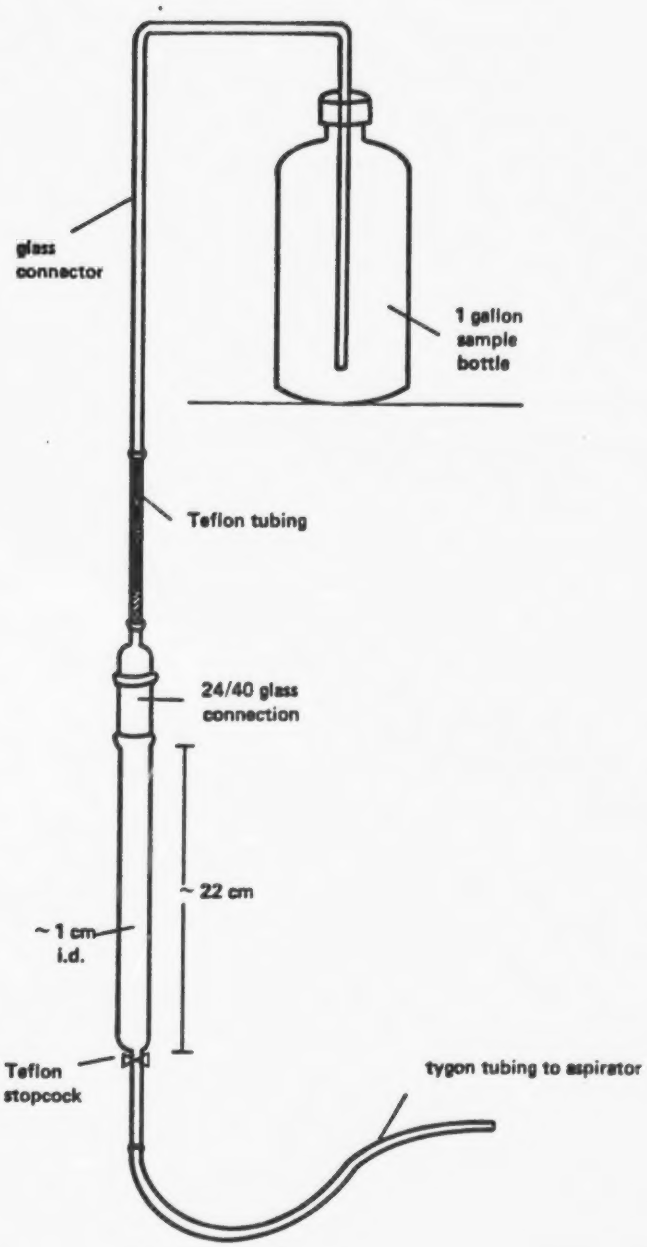

glass connector
1 gallon sample bottle
Teflon tubing
24/40 glass connection
~ 22 cm
~ 1 cm i.d.
Teflon stopcock
tygon tubing to aspirator

Figure 10.2. Chromatography column with siphoning adaptor for drinking water.

(2) One 10 mL graduated pipette with the tip cut off for measuring and transferring resin material.

(3) One graduated cylinder for measuring sample volume. The size of the the cylinder will depend upon the results of conductivity measurements. In general, they may be:

1000 mL for drinking water
500 mL for surface water
250 mL for industrial waste effluent
50 mL for energy wastewater effluents

The cylinder may be reused for each sample if it is rinsed 5 times with deionized water.

(4) One conductivity bridge - range 1 to 30,000 ohms.

(5) Eighteen 250 mL round bottom flasks (24/40).

(6) Five distillation apparatus (Figure 10.3) consisting of:
   (a) one distilling head (Lab Glass ML-135-700).
   (b) one long path condenser (Lab Glass ML-560-700). Jacket of column should be approximately 10 cm in length.
   (c) one condenser adapter (Lab Glass ML-390-700).
   (d) one enlarging adapter (14/24 to 24/40) (Ace Glass 9092-24).
   (e) tygon tubing to connect cooling water source to condenser.
   (f) one 250 mL round bottom flash with 14/24 joint.

(7) Five variable transformers.

(8) Five heating mantles to fit 250 mL round bottom flasks.

(9) Micro-porous boiling chips (Todd Scientific Co. 3006).

(10) One pH meter with a combination electrode.

(11) One rotary evaporator. If more than one is available up to four samples can be evaporated at one time. Samples must be watched closely at the start but after acetone has evaporated, the samples require little attention.

(12) Fifteen small (15 mL) round bottom test tubes with 15 mm screw caps (Supelco 3-3112) and Teflon lined rubber septa (Supelco 3-3115). The specific materials must be used to prevent solvent evaporation during derivatization.

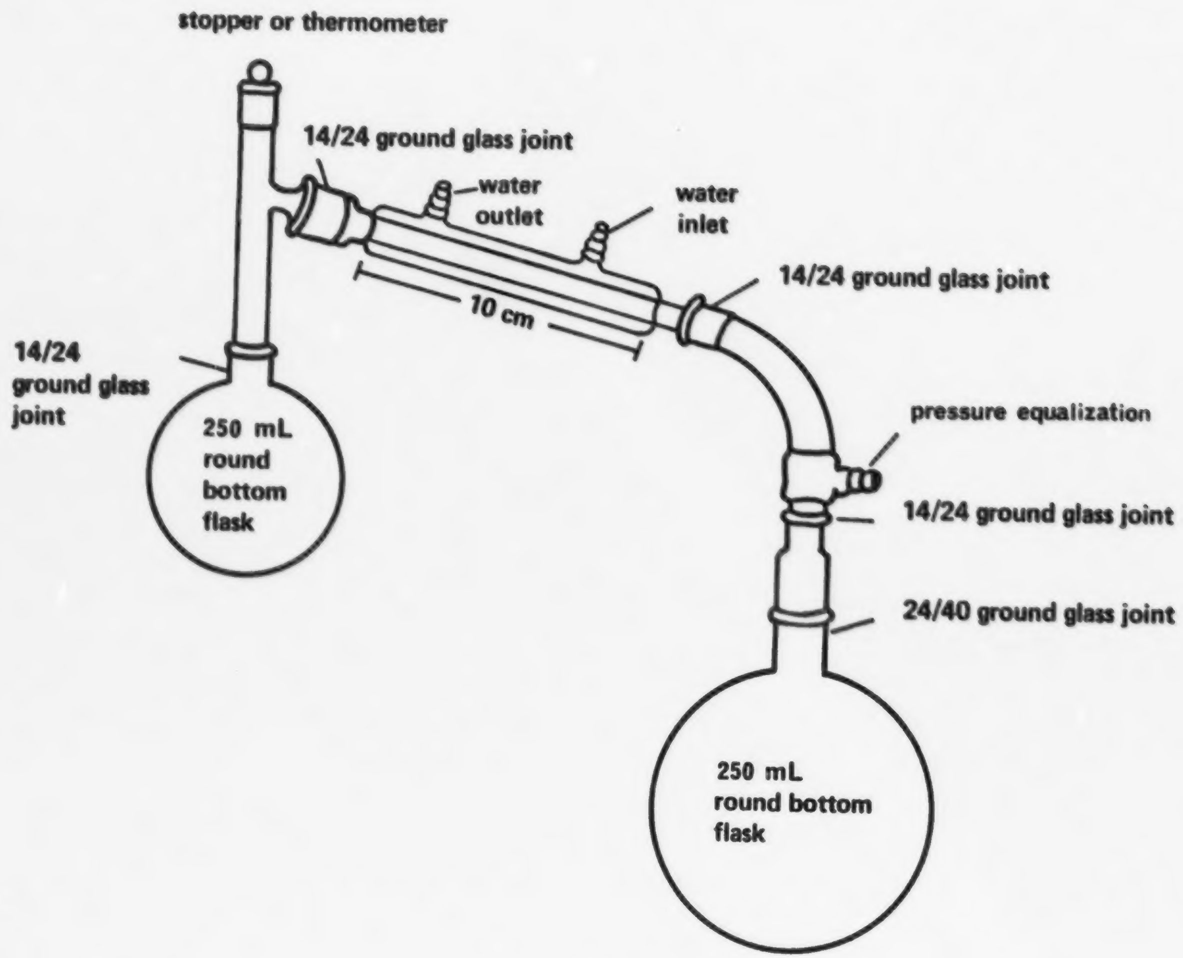

stopper or thermometer
14/24 ground glass joint
water outlet
water inlet
14/24 ground glass joint
10 cm
14/24 ground glass joint
250 mL round bottom flask
pressure equalization
14/24 ground glass joint
24/40 ground glass joint
250 mL round bottom flask

Figure 10.3. Distillation apparatus.

(13) One Soxhlet extractor for cleaning ion exchange resin. A minimum 150 mL capacity is required. Resins can be processed in large batches and stored; therefore a 500 mL to 1000 mL size would be preferred.

(14) Ten mL volumetric flasks for preparing standard solutions.

(15) Fifteen vials (15 mL) with Teflon lined screw caps for storing standard solutions.

(16) Syringes for preparing standard solutions - 50 and 250 μL.

(17) Graduated pipettes for preparing standard solutions - 1 and 2 mL.

(18) One large chromatography column for preparing resin material. The dimensions of this column are not critical, it should hold a minimum of 160 mL of resin material; for large batches of resin, a larger column is preferred. The flow through the column should not exceed 50 mL/minute.

(19) One heating block capable of maintaining 60°C with at least nine spaces for the 15 mL test tubes.

(20) One manifold with a temperature controlled water bath or heating block for nitrogen blowdown. A manifold with nine spaces is preferred but not essential.

(21) One gas chromatograph suitable for capillary column chromatography and all required accessories including syringes, gases, flame ionization detector, and a strip chart recorder.

(22) One 30 m × 0.34 mm I.D. DB-1 (1.0 μ film thickness) fused silica capillary column.

(23) Materials and reagents

  (a) 120 mL AG 1-X8 (50-100 mesh) ion exchange resin (Biorad Laboratories, Richmond CA) in Cl^- form.

  (b) Reagent water - reagent water is described as a water source which does not produce a background interference at the limit of detection, with a conductivity <1 ohm. A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

  (c) 1 L acetonitrile (Burdick and Jackson, pesticide analysis grade).

(d) 1L methanol (Burdick and Jackson, pesticide analysis grade).

(e) 200 mL NaOH - 0.1N in reagent water (use the same solution as specified in Chapter 5, Section 5.2.14).

(f) 200 mL H_2SO_4 - 0.1N in reagent water (use the same solution as specified in Chapter 5, Section 5.2.14).

(g) 100 mL $Na_2S_2O_3$ solution - 0.05 M in reagent water (use the same solution as specified in Chapter 5, Section 5.2.14).

(h) 1.2 L 1N NaOH and 200 mL 0.1N KOH in reagent water.

(i) 2.5 L $NaHSO_4$ - 0.55M in 50:50 acetone:water. This solution should be prepared just prior to use.

(j) 100 mL K_2CO_3 - 1M in reagent water.

(k) 100 mL benzyl bromide reagent - 5% w/v in acetone.

(l) 1 mL MS external standard solution - 2-fluorobiphenyl and 4-fluoroiodotoluene (300 ng/10 µL each) in CH_2Cl_2.

(m) 1 mL GC external standard solution - hexadecane (30 ng/10 µL) in CH_2Cl_2.

(n) 1 mL system performance standard solution (Table 10.1).

10.3 PREPARATION FOR ANALYSIS

10.3.1 Preparation of System Performance Standard

(1) Prepare individual stock standards as specified in Table 10.2.

- solids - accurately weigh 0.120 g of pure material, dissolve in the specified solvent, dilute to volume in a 10 mL volumetric flask.
- liquids - with a 250 µL syringe accurately measure 120 µL of pure liquid, dissolve in the specified solvent, dilute to volume in a 10 mL volumetric flask.

(2) Prepare a secondary neutral standard. With a 1 mL graduated pipette, measure 0.5 mL of each stock standard in group A into a 10 mL volumetric. Dilute to volume using pesticide grade methylene chloride. Transfer into a Teflon sealed screw-cap bottle. Store at 4°C.

(3) Prepare a secondary acid standard. With 2 mL gradaute pipette, measure 0.5 mL of the d_7-butyric acid, 1.65 mL of the hexanoic acid, and 25 µL of the crotonic acid (use a 50 µL syringe) stock standards into a 10 mL volumetric flask. Dilute to volume using

Table 10.1. SYSTEM PERFORMANCE SOLUTION FOR VOLATILE STRONG ACIDS (VOSA)

Compounds	Concentration (ng/µL)
2,6-dimethylphenol	300
acetophenone	310
1-tetradecanol	240
1-octadecene	240
n-octadecane	230
DFTPP	300
n-eicosane	300
pyrene	300
n-heneicosane	300
methyl stearate	10
2-fluorobiphenyl	300
d_7-butyric acid[a] (benzyl ester)	300
crotonic acid[a,b] (benzyl ester)	15
n-hexanoic acid[a,c] (benzyl ester)	1000

[a] Concentration as the underivatized acid in solution.

[b] For determining limit of detection.

[c] For determining capillary capacity.

pesticide grade acetone. Transfer into a Teflon sealed screw cap bottle. Store at 4°C.

(4) Fresh standards should be prepared every six months. If degradation or evaporation has occurred, standards should be prepared sooner.

(5) If compound purity is 96% or greater, the weight or volume can be used without correction to calculate the concentration of stock standards. Compounds which are less than 96% pure cannot be used for standards.

(6) With a 1 mL graduated pipette, measure 0.5 mL of the secondary acid standard into a 15 mL round bottom test tube. Add 0.1 mL of aqueous 1M K_2CO_3. Mix well. Evaporate to dryness using nitrogen blowdown at an elevated temperature (~60°C). Add an

Table 10.2. STOCK STANDARDS FOR SYSTEM PERFORMANCE SOLUTION

Compounds	Density (@ 20°C)	Concentration mg/10 mL
Group A[a]		
2,6-dimethylphenol	S[b]	120
acetophenone	1.033	124
1-tetradecanol	0.823	98
1-octadecene	0.789	95
n-octadecane	0.777	93
DFTPP	S	120
n-eicosane	S	120
pyrene	S	120
n-heneicosane	S	120
methyl stearate	S	120
2-fluorobiphenyl	S	120
Group B[c]		
d_7-butyric acid	S	120
crotonic acid	S	120
n-hexanoic acid	S	120

[a] Solvent is methylene chloride.

[b] S = solid.

[c] Solvent is acetone.

aliquot (0.5 mL) of benzyl bromide reagent to the residue. Cap the tubes, shake, and react the solution overnight at 60°C.

(7) With a 1 mL graduated pipette, add 0.5 mL of the secondary neutral standard to give the SPS as described in Table 10.1. Cap the test tube. Store at 0°C until GC/MS analysis.

10.3.2 Preparation of Resin Material

Ion-exchange resin is cleaned and converted to its OH^- form prior to preparing the resin columns. One hundred and twenty mL of resin material is needed to process 9 samples and 4 procedural blanks and is the minimum amount of resin which should be prepared. The following instructions describe procedures based on 120 mL of resin material; however, it is possible to prepare larger batches and store the prepared resin. If a larger volume of resin is prepared, volumes for rinsing the resin should be adjusted accordingly.

Biorad AG1-X8 (50-100 mesh) in the Cl^- form is extracted with redistilled, pesticide analysis grade methanol in a Soxhlet extractor overnight followed by a 24 hr extraction with redistilled pesticide analysis grade acetonitrile. The resin is placed in a column and rinsed with 10 bed volumes (1.2 L) of deionized water and 20 bed volumes (2.4 L) of 1N NaOH. The resin is then rinsed with reagent water until the eluate water is at pH 6 to 7.

Residual chlorine on the resin material will interfere with sample derivatization. To assure that chlorine and other interferents have been removed a procedural blank should be run using the cleaned resin material prior to processing any samples, as specified in Section 10.3.6.3 and Section 10.4, step 8.

10.3.3 Cleaning of Materials

(1) All glassware to be used is washed with Amway S-A-8 laundry compound (or equivalent) rinsed with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with foil (precleaned with hexane) to prevent contamination.

(2) Teflon liners and Teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. The sonicated liners are vacuum-oven dried for 3 to 5 hours at 70°C, ~28 inches of water, and stored in clean, Teflon lined screw-cap bottles.

10.3.4 Maximum Sample Size

Conductivity measurements must be determined for each sample to assure that the total concentration of anions in the sample does not exceed the resin capacity. Prior to measuring conductivity, check the pH of the sample using a pH meter. Adjust the sample pH to 7.5 to 8.5 using 0.1N KOH or 0.1N H_2SO_4 if necessary. Table 10.3 lists the sample size which should be used for a given conductivity, regardless of water type. Corresponding detection limits are also included.

Table 10.3 ACCEPTABLE SAMPLE SIZE AND CORRESPONDING DETECTION LIMITS FOR SAMPLE CONDUCTIVITY RANGES

Conductivity Range (ohm)	Sample Size (mL)	Detection Limit (ppb)
<150	2500	1
150-300	1000	3
300-600	500	6
600-1,200	250	12
1,200-3,000	100	30
3,000-6,000	50	60
6,000-12,000	25	120
12,000-30,000	10	300
30,000-60,000	5	600

10.3.5 GC/FID Performance Evaluation

Prior to analyzing procedural blanks, acceptable performance for the GC/FID system must be demonstrated.

(1) Analyze the GC/MS system performance standard (Table 10.1) as specified in Table 10.4. Figure 10.4 shows a total ion chromatogram of a similar mixture analyzed by GC/MS under similar conditions that may be used to identify test components in the standard.

(2) Measure peak asymmetry or tailing for acetophenone and 1-tetradecanol using the percent peak asymmetry factor (PAF):

$$\%\ \text{PAF} = \frac{B}{F} \times 100$$

where

B = the width of the back half of a chromatographic peak measured at 10% above baseline

F = width of the front half of the chromatographic peak measured at 10% above baseline.

PAF should measure less than 200% for both acetophenone and 1-tetradecanol.

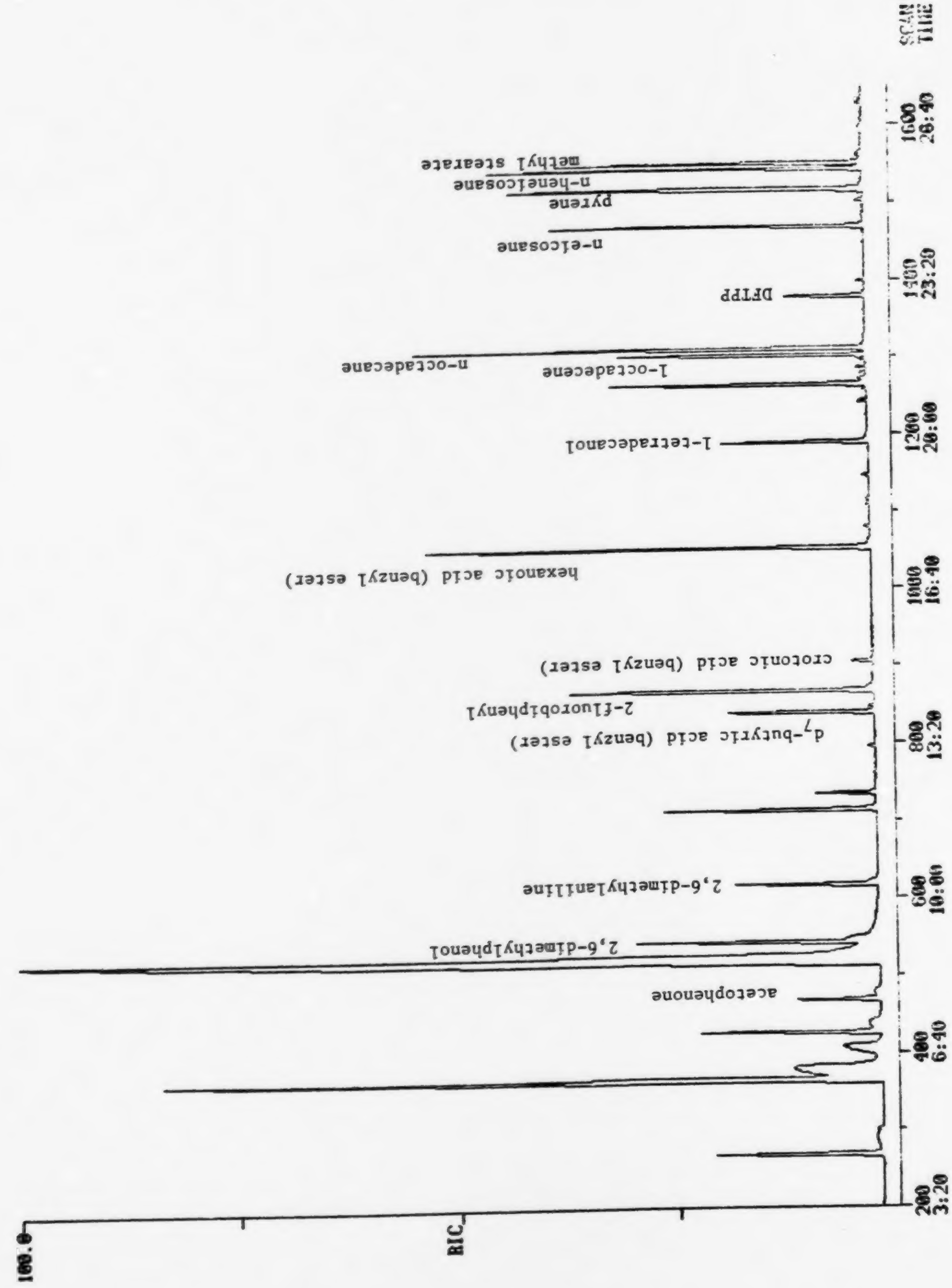


Figure 10.4. Total ion chromatogram for VOSA SPS.

(3) Sensitivity is checked by measuring the signal-to-noise ratio for crotonic acid. A signal-to-noise ratio of 10 to 1 or greater is acceptable.

10.3.6 Derivatization and Procedural Blanks and Controls

Prior to processing any samples a derivatization blank and a derivatization control must be run and analyzed by GC/FID to assure that background contamination during derivatization is low and that derivatization yield is acceptable. After validating the derivatization step, three procedural blanks must be processed and analyzed by GC/FID to detect contamination and artifacts from the resin material, solvents, reagents, dirty glassware, and other sources.

10.3.6.1 Derivatization Blank --

(1) Add 0.1 mL aqueous 1M K_2CO_3 to a 15 mL round bottom test tube. Derivatize as described in step 10.4(10).

(2) With a 50 μL syringe, add 10 μL of the GC external standard (hexadecane) solution to the derivatized solution.

(3) Analyze the solution by GC/FID using the conditions described in Table 10.4. Contaminant (other than the derivatizing reagents) peak heights should be less than 10% relative to the peak height of the external standard, hexadecane.

(4) If significant contamination is present, rerun derivatization blanks using fresh glassware and reagents (acetone, benzyl bromide, and K_2CO_3). Reagents may be distilled or taken from another source.

10.3.6.2 Derivatization Control --

(1) Prepare a control solution. With a 50 μL syringe, measure 25 μL of each stock solution in group B (Table 10.2) into a 10 mL volumetric flask and dilute to volume using pesticide grade acetone. (Unlabeled butyric acid may be used instead of d_7-butyric acid.) Transfer into a Teflon sealed screw cap bottle and store at 4°C.

(2) With a 1 mL graduated pipette, measure 0.5 mL of this control solution into a 15 mL round bottom test tube. Add 0.1 mL of aqueous 1M K_2CO_3. Mix well. Evaporate to dryness using nitrogen

Table 10.4. GC/FID OPERATING CONDITIONS FOR VOLATILE STRONG ACIDS

GC Column	30 m DB-1 (1.0 μ film thickness) fused silica, wide bore (0.34 mm i.d.), capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through the column; 15:1 split injection
Temperature Program	80 to 250°C @ 5°/min
Injector Temperature	250°C
Detector Temperature	260°C
Injection Volume	1.0 μL

blowdown at an elevated temperature (~60°C). With a 1 mL graduated pipette, add an aliquot (0.3 mL) of benzyl bromide reagent to the residue. Cap the tube, shake, and react the solution overnight at 60°C.

(3) With a 50 μL syringe, add 10 μL of the GC external standard to the derivative mixture.

(4) Analyze samples by GC/FID as described in Table 10.4. Peak heights of the three control acid derivatives measured relative to the external standard should fall within the range of 0.9-1.1.

(5) If derivative yield is low (as measured by relative peak heights), this step must be repeated using fresh reagents and glassware. Reagents may be distilled or taken from another source.

10.3.6.3 Procedural Blanks --

For each batch of materials and reagents that are used, a set of three procedural blanks are required. Table 10.5 identifies these blanks and defines their purpose. These blanks must be processed and analyzed by GC/FID prior to processing any samples. If a large number of samples are to be processed, it is advantageous to prepare large batches of materials and reagents thereby reducing the number of blanks which must be run.

Table 10.5. PROCEDURAL BLANKS

Blank	Description
Procedural Blank 1	Detects contamination in resin material, solvents, and glassware
Procedural Blank 2	Detects contamination in reagent water
Procedural Blank 3	Detects contamination in sulfuric acid, sodium hydroxide, and sodium thiosulfate solutions used during sample collection and analysis

Procedural blank 1 must also be run every time a set of samples is processed and analyzed.

10.3.6.3.1 Procedural Blank 1--

(1) Prepare a resin column as described in step 10.4(1).

(2) Follow the procedure for volatile acid analysis as described in steps 10.4(5) to 10.4(10).

(3) If more than 3 mL of the K_2CO_3 solution is needed to neutralize the distillate, residual chlorine has not been removed from the resin material. Additional rinsing with 1N NaOH is required before using the resin material.

(4) With a 50 μL syringe, add 10 μL of the GC external standard to each derivatized mixture.

(5) Analyze samples by GC/FID as described in Table 10.4. Contaminant (other than the derivatizing reagent, benzyl acetate, and benzyl propionate) peak heights should measure less than 25% relative to the standard.

(6) If significant contamination is present, the procedure should be repeated using a blank chromatography column with no resin added. If this blank is acceptable, then the resin is the source of contamination and should be recleaned using the procedure in Section 10.3.2. A procedural blank 1 should then be repeated using the fresh resin material.

(7) If significant contamination is present in this blank, fresh reagents and clean glassware should be used and the reagent blank repeated. When the reagent blank is acceptable, a procedural blank 1 must be repeated. Procedural blanks 2 and 3 may be processed once the procedural blank 1 is acceptable.

10.3.6.3.2 Procedural Blanks 2 and 3--

(1) Prepare a resin column as described in step 10.4(1). Attach a separatory funnel to the top of the column. For procedural blank 2, pour 100 mL of reagent water into the separatory funnel, then drain the sample through the resin bed at a flow rate of ~10 mL/ minute. For procedural blank 3, use 100 mL of reagent water spiked with 1 mL each of the sulfuric acid, sodium hydroxide, and sodium thiosulfate solutions. These must be the same solutions which are to be used when the samples are collected (Chapter 5, section 5.2.14).

(2) Follow the extraction procedure as described in steps 10.4(5) to 10.4(10).

(3) With a 50 µL syringe, add 10 µL of the GC external standard to each derivative mixture.

(4) Analyze the samples by GC/FID using the conditions in Table 10.4. Peak heights for contaminants in procedural blank 3 which are not present in procedural blank 2 should measure less than 20% relative to the external standard.

(5) If significant contamination is present in Procedural blank 3 which is not present in procedural blank 2, the procedure should be repeated using fresh solutions of sulfuric acid, sodium hydroxide, and sodium thiosulfate until an acceptable blank is achieved.

10.4 ION-EXCHANGE SEPARATION AND DERIVATIZATION

(1) Prepare ion exchange column by placing a small glass wool plug in the bottom of the chromatography column (Fig. 10.1). Using a graduated pipette with the tip cut off, pipette 10 mL (measure volume after resin has settled) of cleaned Biorad AG 1-X8 resin into the open tubular chromatography column, allow the resin to settle, and rinse resin bed with ~10 mL of reagent water.

(2) If the sample is turbid, allow it to stand undisturbed for 4 hours or overnight prior to analysis to allow particulates to settle.

(3) Gently pour the aqueous portion of the sample (equilibrated to room temperature) into a graduated cylinder to measure sample volume. Both total volume of sample collected and the volume of sample analyzed should be recorded.

(4) For surface waters and effluents, transfer sample water to the reservoir on top of the column and allow it to drain through the resin bed. Flow is gravity controlled and should be approximately 5 mL/min (NOTE: The glass wool plug can restrict flow if packed too tightly.) For drinking water, transfer sample to a one gallon container. Attach siphoning adaptor (Fig. 10.2) between sample container and column, and drain sample through the resin bed. Attach a piece of tygon tubing between the end of the chromatography column and a water aspirator. To start sample flowing through the resin, open the stopcock on the chromatography column and turn on the aspirator. This should create sufficient suction to pull the water sample through the siphon adaptor and onto the resin column. Disconnect suction after water is flowing through the resin bed. Using the stopcock at the base of the column, adjust flow through the column to ~2.5 mL/minute. At this flow, it will require ~16 hours for the sample to drain through the resin bed, which is suitable for overnight accumulation. If particulate matter in the sample plugs the column, positive head pressure can be used to restore flow.

(5) Rinse the resin with 25 mL of deionized water. Flow is gravity controlled (~5 mL/minute).

(6) Elute organics from the column using 150 mL of 0.55M $NaHSO_4$ in acetone:water (1:1). Acids should be eluted immediately after all of the sample has passed through the resin column. Collect the eluant solvent in a 250 mL round bottom flask. Add a boiling chip to the flask.

(7) Connect the flask containing the eluant solution to the distillation apparatus (Figure 10.3). Heat the flask and allow the distillation to proceed until all liquid has distilled into the 250 mL round bottom flask. Distillation should be complete in ~30 minutes. The sample should be heated gradually to avoid bumping at the beginning of the distillation. Samples should be distilled immediately after elution.

(8) Volatile acids in the distillate are converted to their nonvolatile salts by adding sufficient aqueous 1M K_2CO_3 to the distillate to raise the pH to 8 or higher. (If more than 2 mL of the K_2CO_3 solution is needed to raise the pH of the procedural blank, residual chloride has not been removed from the resin material, see Section 10.3.6.3.1). Samples can be stored refrigerated anytime after this step is complete.

(9) Concentrate the distillate to ~2 mL using rotary evaporation at an elevated temperature (~60°C). Some industrial effluents may foam during rotary evaporation. If this occurs the entire sample may be concentrated using nitrogen blowdown at an elevated temperature (~70°C).

(10) Transfer the concentrate to a 15 mL, round bottom, screw cap, test tube. Rinse the evaporation flask with two 2.5 mL portions of distilled water and combine with the concentrate. Evaporate the concentrate to dryness using nitrogen blowdown at an elevated temperature (~60°C). With a 1 mL graduated pipette, add an aliquot (0.3 mL) of benzyl bromide reagent solution to the residue. The tubes are capped, shaken, and the solution is allowed to react at 60°C overnight. Since the derivatizing reagent is the solvent, no further manipulations are needed. If samples evaporate to dryness during derivatization, they must be discarded. If they partially evaporate, adjust to a known volume before GC/MS analysis.

(11) After derivatization add 10 µL of the MS external standard solution to each derivatized sample concentrate using a 50 µL syringe. Store the derivatized samples at 0°C until GC/MS analysis.

10.5 GC/MS/COMP ANALYSIS

Prior to beginning GC/MS/COMP analysis of the volatile strong acids fraction the analyst should be familiar with the procedures described in Chapter 13, "GC/MS/COMP Analysis - General for all Protocols."

10.5.1 Preparation for Analysis

10.5.1.1 GC/MS/COMP Operating Parameters--

The recommended GC/MS operating parameters are given in Table 10.6 for the analysis of volatile strong acids (VOSA). Other GC capillaries which meet the system performance criteria may be substituted for the recommended fused silica (See Section 13.4). It is also recommended that the capillary used here be used only with sample extracts containing methyl esters of strong acids.

10.5.1.2 MS Calibration--

The mass spectrometer is calibrated using the manufacturer's recommended approach (Chapter 13). The acceptability of the calibration results is verified with the analysis of the system performance solution (SPS).

Table 10.6. GC/MS OPERATING CONDITONS FOR VOLATILE STRONG ACIDS AS THEIR BENZYL ESTERS

GC Column	30 m DB-1 (1.0 μ film thickness) fused silica wide bore (0.34 mm I.D.) capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through the column; 9:1 split injection
Carrier Gas Sweep Time	85 sec (50°C)
Temperature Program	65°C/5 min; to 250°C @ 4°/min and hold
Injector Temperature	250°C
Transfer Line Temperature	255°C
Injection Mode	Splitless 0.4 min/split
Injection Volume	1.0 μL
Ionizing Energy	70 eV
Ion Source Temperature	250°C
Scan Range	35-500
Scan Rate	0.9 s, 0.1 sec hold

10.5.2 Analysis of SPS (Quality Control)

Using the prescribed GC/MS conditions the SPS is analyzed and all necessary data is acquired and evaluated prior to proceeding to sample analysis or determination of RMRs. The SPS is analyzed at the beginning of each day's operation.

The SPS raw data is extracted from the run, test parameters calculated and plotted or tabulated (Tables 10.7 and 10.8). Up to 14 days of SPS analytical results can be historically recorded in Table 10.7 for day-to-day comparisons. This allows the analyst to anticipate GC/MS maintenance requirements. Calculation and usuage of these data are discussed in Chapter 13.

As part of additional QC/QA procedures, a check of RMRs is made on a daily basis (Table 10.8). The RMRs are compared to those obtained during the development of the historical data bank. The usage of these data is also discussed in Chapter 13.

10.5.3 Determination of RMRs

RMRs for the User's data bank (as opposed to the daily RMR checks discussed in the above paragraph) are determined by injecting a solution containing the derivatized internal standards and analytes.

The GC/MS/COMP operating parameters are given in Table 10.6. Table 13.8 may be used to tabulate RMR raw data.

10.5.4 Analysis of Field and Quality Control Samples

After all specified criteria for GC and MS performance (Tables 10.7 and 10.8) have been met, analysis of VOSA sample extracts is conducted.

A set of VOSA sample extracts may have been derived from procedural blanks, laboratory and field controls and blanks, surrogates, and collected field samples, and are generally analyzed in that respective order.

Qualitative and quantitative procedures are described in Chapter 13. Table 10.9 may be used to tabulate sample analyte raw data prior to using the MASQUANT software program or manual calculations.

Table 10.7. GC-MS SYSTEM PERFORMANCE TEST FOR VOLATILE STRONG ACIDS (VOSA)

Dates: ________ ________

Run Id Code ________ ________

GC Column and Program: ____________________

Notebook References: ____________________

MS SYSTEM CHECKS

High/Low Mass Balance Ion	DFTPP Relative Abundance Criteria
51	30% to 60% of m/z 198
68	<2% of m/z 69
70	<2% of m/z 69
127	40% to 60% of m/z 198
197	<1% of m/z 198
198	100%
199	5-9% of m/z 198
275	10% to 30% of m/z 198
365	at least 1% of m/z 198
441	present, but < m/z 443
442	>40% of m/z 198
443	17% to 23% of m/z 442

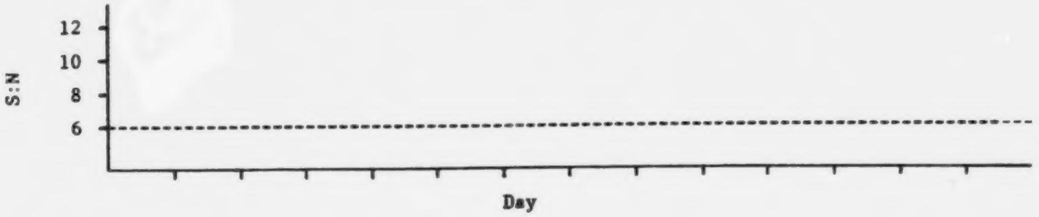
m/z	51	68	70	127	197	198	199	275	365	331	442	443	Pass/ Fail	Remedial Action
Abundance* Criteria	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )		
Day														

*These abundances are initially established by the MAS user, and subsequently become guidelines for acceptability of tune.

(continued)

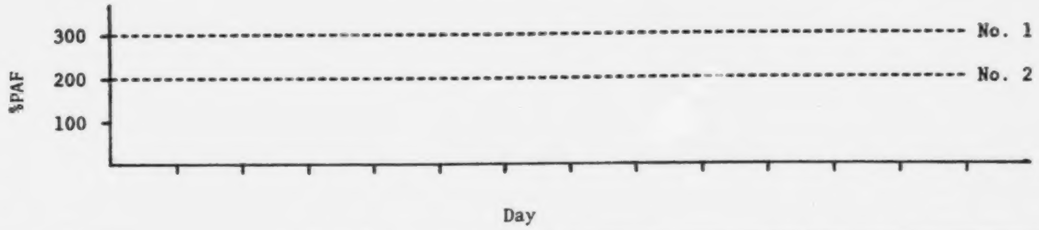
Table 10.7 (cont'd.)

Test	Components	Criteria
Limit of Detection	Crotonic Acid (Benzyl Ester)	S:N (m/z 91) > 6:1

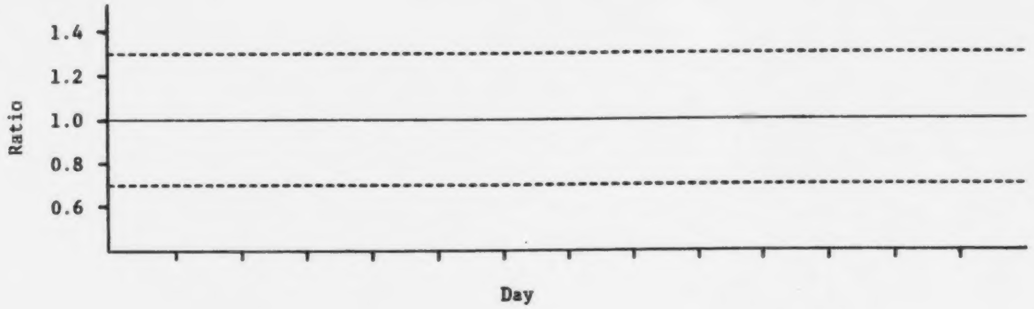


GC SYSTEM CHECKS

Peak Asymmetry	1. Acetophenone (TIC) ● 2. 1-Tetradecanol (TIC) ■	%PAF <300 %PAF <200



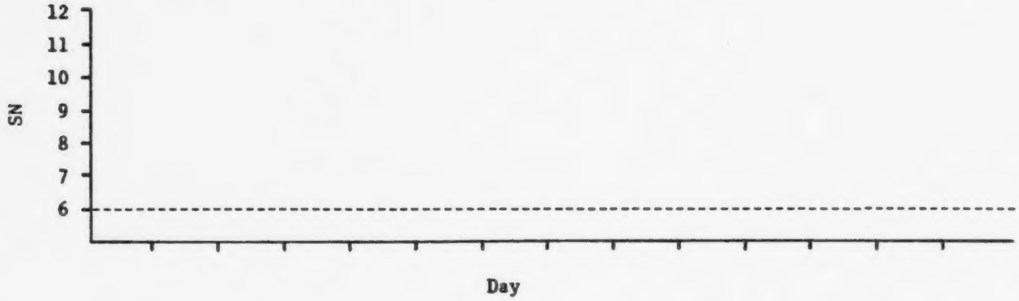
Basicity	1. Acetophenone (TIC) 2. 2,6-Dimethylphenol (TIC)	Ratio 2:1 = 0.7 to 1.3



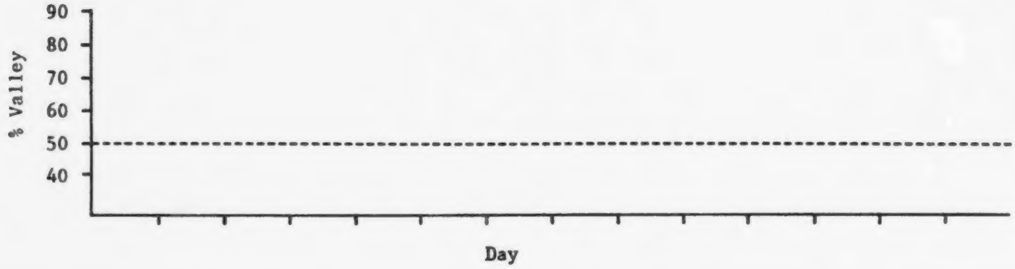
(continued)

Table 10.7 (cont'd.)

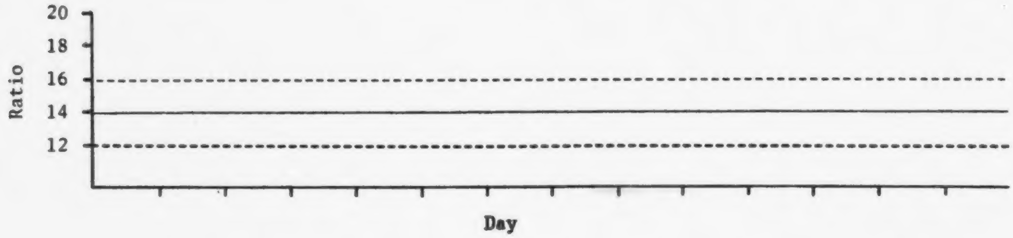
Test	**Components**	**Criteria**
Separation Number	**n-Eicosane (m/z 43)** **n-Heneicosane (m/z 43)**	**SN ≥ 6**



Test	**Components**	**Criteria**
Resolution	n-Octadecane (TIC) 1-Octadecene (TIC)	**R = minimum 50% Valley**



Test	**Components**	**Criteria**
Inertness	**Methyl Stearate**	**Ratio m/z 74 to 298 = 12:1 to 16:1**



(continued)

Table 10.7 (cont'd.)

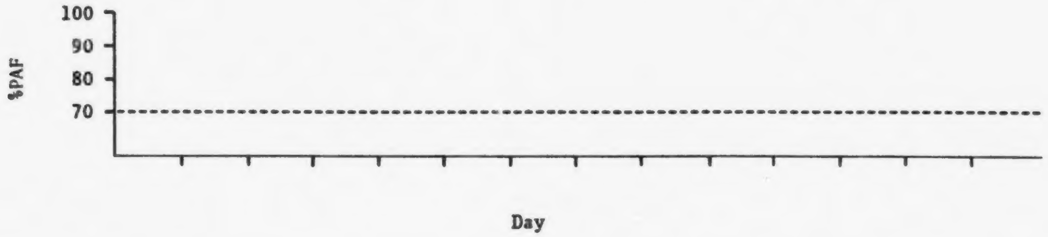
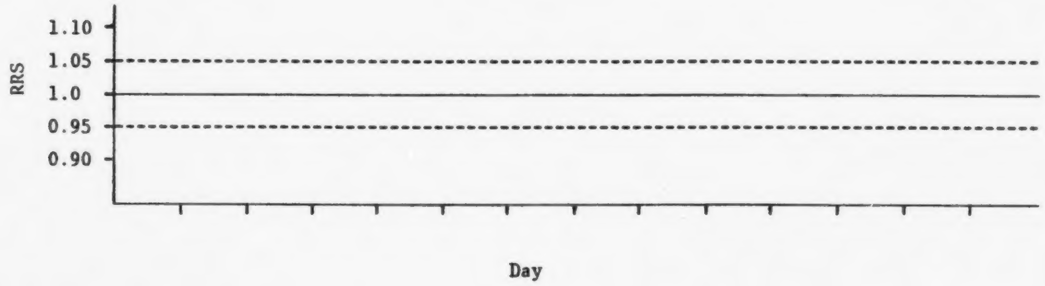
Test	Components	Criteria
Capillary Capacity	Hexanoic Acid (Benzyl Ester) (TIC)	%PAF >70
		
Relative Retention Shift	1. Heneicosane 2. Pyrene	RRS = 0.95-1.05
		

Table 10.8. GC-MS SYSTEM PERFORMANCE TEST: RMR CHECK FOR VOLATILE STRONG ACIDS (VOSA)

Date: ____________________ Run ID Code: ____________________

DATA

Standard	MW	Amount (ng)	μM	Ion (m/z)	Area (Run 1)	Area (Run 2)
2-Fluorobiphenyl	172			172		
d_7-butyric acid[a]	109			50		
				109		

MATRIX OF STANDARD ION RMRs

Standard	Ion	172	50	109
2-Fluorobiphenyl	172	-		
d_7-butyric acid[a]	50		-	
	109			-

[a] As methyl esters.

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

Table 10-9. **RAW ANALYTE DATA FOR VOSA FRACTION**

Date: ______________________ Run I.D. Code: ______________

Sample Identification: _______ Notebook Reference: __________

Vol. Water (L): _____________ Vol. Water Processed (mL): ____
(to which IS were added)

STANDARDS

	I.D. No.	Spectrum No.	Weight (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
2-Fluorobiphenyl				172:	______,______	___	___,___
d_7-Butyric Acid				50,109:	______,______	___	___,___

ANALYTES Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Area	MW_x^a
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___
______________	____	________	___,___:	______,______	___

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{RMR_{x/y}} \times \frac{ng_y}{A_y \cdot MW_y}$$

$$K = \frac{ng_y}{A_y \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

CHAPTER 11

ANION-EXCHANGE AND ANALYSIS OF NONVOLATILE ACIDS (NOVA)

11.1 INTRODUCTION

11.1.1 Principle of the Method

This method describes the determination of nonvolatile organic acids in drinking water, surface waters, and effluents. Since strong acids such as strong phenols and sulfonic, phosphonic and dicarboxylic acids are too ionic to efficiently extract from water into organic solvents, the procedure utilizes ion-exchange resins to concentrate them from the aqueous matrix. Acids are eluted from the resin using HCl in methanol. The mineral acid protonates the organic acids to their nonionic forms, which are then rinsed off the resin with methanol. Both HCl and methanol are removed using rotary evaporation without significant losses of the nonvolatile acids. These organic acids must be derivatized prior to gas chromatography in order to increase compound volatility and reduce adsorptive losses. Diazomethane was the only reagent tested which successfully derivatized sulfonic and phosphonic acids to chromatographable products. Since diazomethane will methylate only free acids, a small volume of HCl:methanol is added to sample concentrates just prior to derivatization. The samples are concentrated using nitrogen blowdown to remove excess derivatizing reagent, HCl and solvent.

11.1.2 Detection Limits

If an average detection limit for GC/MS analysis for methylated nonvolatile acids is estimated as 10 ng per component, and the final volume of derivatized sample is 0.5 mL, then nominal detection limits for a 1 μL injection are directly related to sample size:

2 ppb for a 2.5 L sample (drinking water);
10 ppb for a 500 mL sample (surface water);
20 ppb for a 250 mL sample (industrial and municipal wastewater); and
100 ppb for a 50 mL sample (energy effluents).

11.1.3 Interferences

Background from thermal decomposition of humic acids which are extracted from the sample by the ion-exchange resin during gas chromatographic analysis may cause interferences for surface waters and municipal wastewater effluents. A procedural blank must be run prior to processing any samples to assure that contamination and artifacts from resin material, solvents, reagents, glassware, and other sources is low.

11.1.4 Precision, Accuracy, and Scope

Table 1.8 in Chapter 1 presents average recoveries and standard deviations for a variety of organic acids. This method gives acceptable recoveries for sulfonic, dicarboxylic, and certain aromatic carboxylic acids, as well as pentachlorophenol. Long chain fatty acids are not recovered well.

11.2 APPARATUS AND REAGENTS

The following materials are required for processing a set of nine samples plus three procedural blanks. Nine is the maximum number of samples (including field and quality control) which should be processed at a time. The corresponding quality control samples which should be run during these analyses are listed in Table 5.1, Chapter 5. The three procedural blanks must be run before any samples are collected or processed. It will require approximately one working day to process nine samples.

(1) Nine glass chromatography columns, ~1 cm I.D. × 22 cm, equipped with 500 mL sample reservoir (Figure 11.1) and a ground glass stopper. The size of the sample reservoir is not critical; however a 500 mL reservoir will hold the entire sample for all water types except drinking water. For drinking water, an adaptor (Figure 11.2) for siphoning the sample onto the resin should be used in lieu of a sample reservoir. This allows unattended flow through the sample for a 14 to 16 hour period such that the sample can be concentrated overnight. A vacuum aspirator is needed to start sample flow through the siphon adaptor and resin column.

(2) One 10 mL graduated pipette with the tip cut off for measuring and transferring resin material.

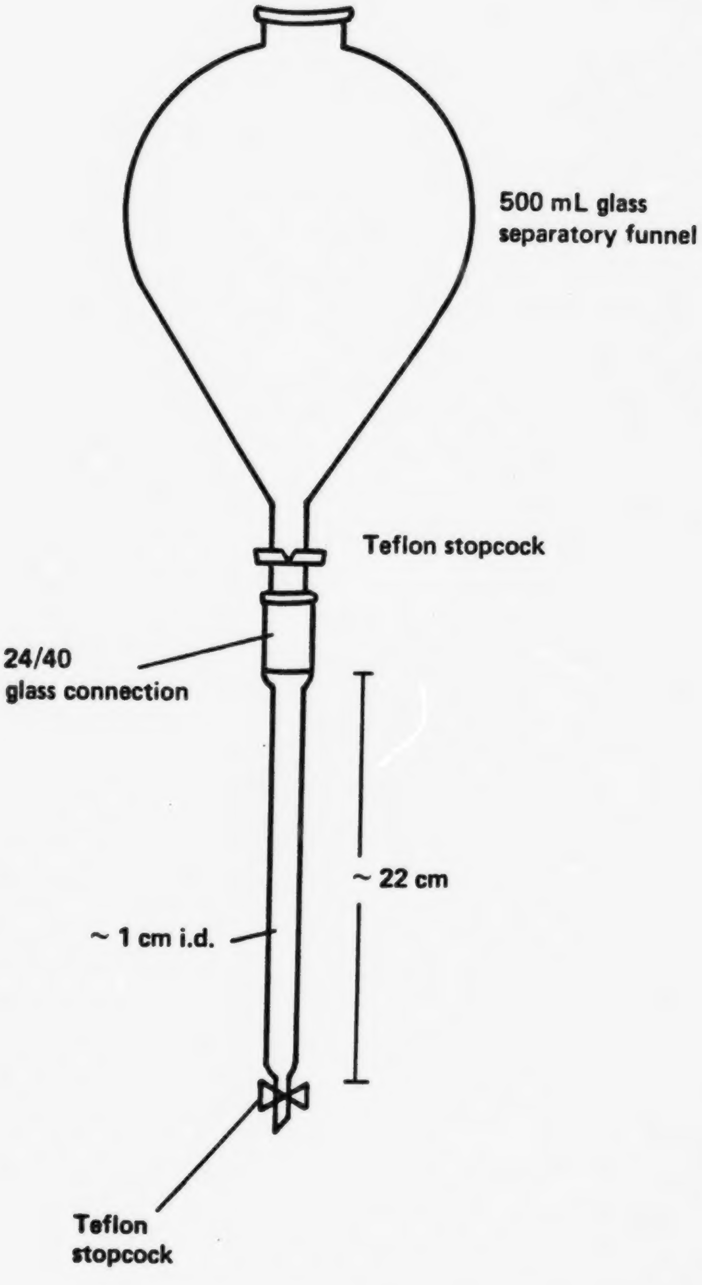

500 mL glass
separatory funnel
Teflon stopcock
24/40
glass connection
~ 22 cm
~ 1 cm i.d.
Teflon
stopcock

Figure 11.1. Chromatography column with sample reservoir (exact dimensions of column are not critical - must hold 10 mL of resin).

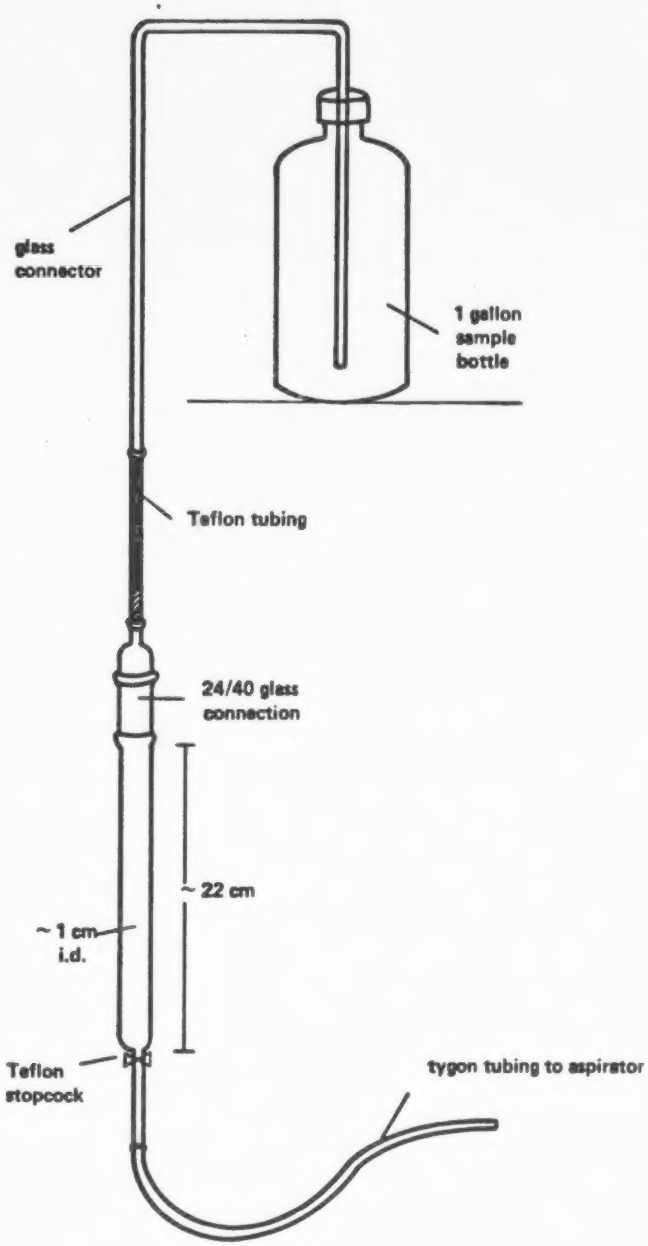

glass connector
1 gallon sample bottle
Teflon tubing
24/40 glass connection
~ 22 cm
~ 1 cm i.d.
Teflon stopcock
tygon tubing to aspirator

Figure 11.2. Chromatography column with siphoning adaptor for drinking water.

(3) One graduated cylinder for measuring sample volume. The size of the sample, thus, the cylinder will depend upon the results of conductivity measurements. In general they may be:

1000 mL for drinking water;
500 mL for surface water;
250 mL for industrial and municipal wastewater effluents; and
50 mL for energy samples.

The cylinder may be reused for each sample if it is rinsed 5 times with deionized water.

(4) One conductivity bridge - range 1 to 30,000 ohm.

(5) Nine 200 mL round bottom flasks (24/40).

(6) One rotary evaporator. If more than one is available up to four samples can be evaporated at a time. Care should be taken to avoid bumping; however since concentrates can go to dryness, the samples require little attention.

(7) One vacuum aspirator for pulling water through the resin column.

(8) Fifteen 15 mL round bottom centrifuge tubes with Teflon lined screw caps.

(9) One Soxhlet extractor for cleaning ion-exchange resin. A minimum 200 mL capacity is required. Resin can be processed in large batches and stored; therefore a 500 mL to 1000 mL size would be preferred.

(10) One large chromatography column for preparing resin material. The dimensions of this column are not critical, it should hold a minimum of 120 mL of resin material; for large batches of resin, a larger column is preferred. The flow through the column should not exceed 50 mL/minute.

(11) One manifold with a temperature controlled water bath or heating block for nitrogen blowdown. A manifold with nine spaces is preferred but not essential.

(12) One pH meter with a combination electrode.

(13) 10 mL volumetric flasks for preparing standard solutions.

(14) 15 mL vials with Teflon lined screw caps for storing standard solutions.

(15) Syringes for preparing standard solutions - 50 and 250 μL.

(16) Graduated pipettes for preparing standard solutions - 1 mL.

(17) One well ventilated hood or toxic lab for working with diazomethane.

(18) Diazomethane generation/distillation apparatus (Aldrich Macro Diazald set Z10,851-0) with all clear glass seals (Caution: the presence of ground glass or scratched glass may catalyze an explosive reaction of dizaomethane).

(19) One gas chromatograph suitable for capillary column chromatography with flame ionization detection and all required accessories including syringes, gases, and a strip chart recorder.

(20) One 30 m × 0.34 mm I.D. DB-5 (1.0 μ film thickness) fused silica capillary column.

(21) Materials and reagents

  (a) 120 mL AG 1-X8 (50-100 mesh) ion exchange resin (Biorad Laboratories, Richmond, CA) in Cl^- form.

  (b) Reagent water - reagent water is described as a water source that does not produce a background interference at the limit of detection and that has a conductivity of <1 ohm. A water purification system (Millipore Super Q or equivalent) may be used to generate reagent water.

  (c) 1 gallon methanol (Burdick and Jackson, pesticide analysis grade).

  (d) one lecture bottle containing gaseous HCl.

  (e) 1 L methyl-t-butyl ether (Burdick and Jackson, pesticide analysis grade).

  (f) 5.0 L 1N KOH in reagent water.

  (g) 240 mL 1N formic acid in reagent water.

  (h) 250 mL 0.1N NaOH in reagent water (use the same solution as specified in Chapter 5, section 5.2.14).

  (i) 250 mL 0.1N HCl in reagent water (use the same solution as specified in Chapter 5, section 5.2.14).

  (j) 100 mL 0.05M $Na_2S_2O_3$ in reagent water (use the same solution as specified in Chapter 5, section 5.2.14).

  (k) 5 mL phenolphthalein solution (0.05 g in 50 mL ethanol + 50 mL reagent water).

(l) 50 mL diethyl ether (Mallinkrodt, pesticide analysis grade, peroxide free).

(m) ~100 mg p-tolylsulfonyl methylnitrosamide (Diazald®, Aldrich Co.).

(n) 10 mL 60% KOH (w/v). Dissolve 6 g KOH in 10 mL reagent water.

(o) 1 mL MS external standard solution - 4-fluoro-2-iodotoluene (300 ng/10 μL) and 2-fluorobiphenyl (300 ng/10 μL) in CH_2Cl_2.

(p) 1 mL GC external standard solution - hexadecane (30 ng/10 μL) in CH_2Cl_2.

(q) 1 mL system performance standard solution for nonvolatile organic acids (Table 11.1).

Table 11.1. GC/MS SYSTEM PERFORMANCE SOLUTION FOR NONVOLATILE ACIDS (NOVA)

Compound	Concentration (ng/μL)
2,6-dimethylphenol	300
acetophenone	310
1-tetradecanol	240
1-octadecene	240
n-octadecane	230
DFTPP	300
n-eicosane	300
pyrene	300
n-heneicosane	300
methyl stearate	300
dimethyl adipate	1000[a]
methyl 2,4,5-trichlorophenoxyacetate	18[a]
2-fluorobiphenyl	300
4-fluoro-2-iodotoluene	130
2-naphthalene-sulfonic acid-d_7, methyl ester	300[a]

[a] Concentration of underivatized acid.

11.3 PREPARATION FOR ANALYSIS

11.3.1 Preparation of System Performance Standard

(1) Prepare individual stock standards as specified in Table 11.2.

- solids - accurately weight 0.120 g of pure material, dissolve in the specified solvent, dilute to volume in a 10 mL volumetric flask.

Table 11.2. STOCK STANDARD SOLUTIONS

Compounds	Density (@ 20°C)	Concentration mg/10 mL
Group A[a]		
2,6-dimethylphenol	S[b]	120
acetophenone	1.030	124
1-tetradecanol	0.823	98
1-octadecene	0.789	95
n-octadecane	0.777	93
DFTPP	S	120
n-eicosane	S	120
pyrene	S	120
n-heneicosane	S	120
methyl stearate	S	120
2-fluorobiphenyl	S	120
4-fluoro-2-iodotoluene	0.883	110
Group B[c]		
2-naphthalene-sulfonic acid-$d_7 \cdot H_2O$	S	120
2,4,5-trichlorophenoxyacetic acid	S	120
adipic acid	S	400

[a] Solvent is methylene chloride.

[b] S = solid.

[c] Solvent is methanol.

- liquids - using a 250 μL syringe, accurately measure 120 μL of pure liquid, dissolve in the specified solvent, dilute to volume in a 10 mL volumetric flask.

(2) Prepare a secondary neutral standard. With a 1.0 mL graduated pipette measure 0.5 mL of each stock standard in group A (for 4-fluoro-2-iodotoluene measure 0.25 mL) into a 10 mL volumetric flask. Dilute to volume using pesticide grade methylene chloride. Transfer into a Teflon sealed screw cap bottle. Store at 4°C.

(3) Prepare a secondary acid standard. With a 1.0 mL graduated pipette measure 0.5 mL of the group B standards (for 2,4,5-trichlorophenoxy acetic acid measure 30 μL with a 50 μL syringe) into a 10 mL volumetric flask. Dilute to volume using pesticide quality methanol. Transfer into a Teflon sealed screw cap bottle. Store at 4°C.

(4) Transfer the stock standards into Teflon sealed screw cap bottles. Store at 4°C.

(5) Fresh standards should be prepared every six months. If degradation or evaporation has occurred, standards should be prepared sooner.

(6) If compound purity is 96% or greater, the weight or volume can be used without correction to calculate the concentration of stock standards. Compounds which are less than 96% pure cannot be used for standards.

(7) With a 1 mL graduated pipette, measure 0.5 mL of the secondary acid standard into a 15 mL round bottom test tube. Evaporate to ~0.1 mL using nitrogen blowdown at 40°C. Resuspend the sample in 0.2 mL of 1$\underline{N}$ HCl:methanol solution (See Section 11.3.4 for preparation of HCl:methanol).

(8) Derivatize the sample using diazomethane as described in Section 11.4.2, steps 1 to 10.

(9) Evaporate the sample to 0.5 mL using nitrogen blowdown at 30°C. With a 1 mL graduated pipette, add 0.5 mL of the secondary neutral standard to the derivatized mixture and mix well. This gives the concentrations shown in Table 11.1. Cap the test tube. Store at 4°C until GC/MS analysis.

11.3.2 Preparation of Resin Material

Ion-exchange resin is cleaned prior to preparing the resin columns. One hundred and twenty mL of resin material is needed to process 9 samples and three procedural blanks and is the minimum amount of resin which should be prepared. The following instructions describe procedures based on 120 mL of resin material; however, it is possible to prepare larger batches and store the prepared resin wet in a sealed glass jar. If a larger volume of resin is prepared, volumes for rinsing the resin should be adjusted accordingly.

Biorad AG 1-X8 (50-100 mesh) in the Cl^- form is extracted with pesticide grade methanol in a Soxhlet apparatus overnight. The resin is placed in a column and rinsed with 10 bed volumes of deionized water (1.2 L), 20 bed volumes of 1N NaOH (2.4 L), and 2 bed volumes of 1N formic acid (240 mL). The resin is then rinsed with deionized water until the eluant water is at pH 5 to 6. Resin which is not used immediately may be stored wet, sealed in glass jars.

11.3.3 Cleaning of Materials

(1) All glassware to be used is washed with Amway S-A-8 laundry compound (or Isoclean or other nondetergent) rinsed with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with foil (precleaned with hexane) to prevent contamination.

(2) Teflon liners and teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. The sonicated liners are vacuum-oven (~20 inches of water) dried for 3 to 5 hours at 70° and stored in clean, Teflon lined screw-cap bottles.

11.3.4 Preparation of Gaseous HCl:Methanol

1N HCl:methanol solution is usually prepared in large batches and stored in the refrigerator for up to 2 weeks between use. Because HCl is corrosive, the entire operation should be performed in a well-ventilated hood. Pesticide analysis grade methanol (500 mL) is added to a 1 L amber glass bottle and the bottle is chilled in an ice bath. Gaseous HCl is bubbled through the methanol as illustrated in Figure 11.3. HCl is allowed to dissolve into the methanol for ~15 minutes. The HCl concentration of

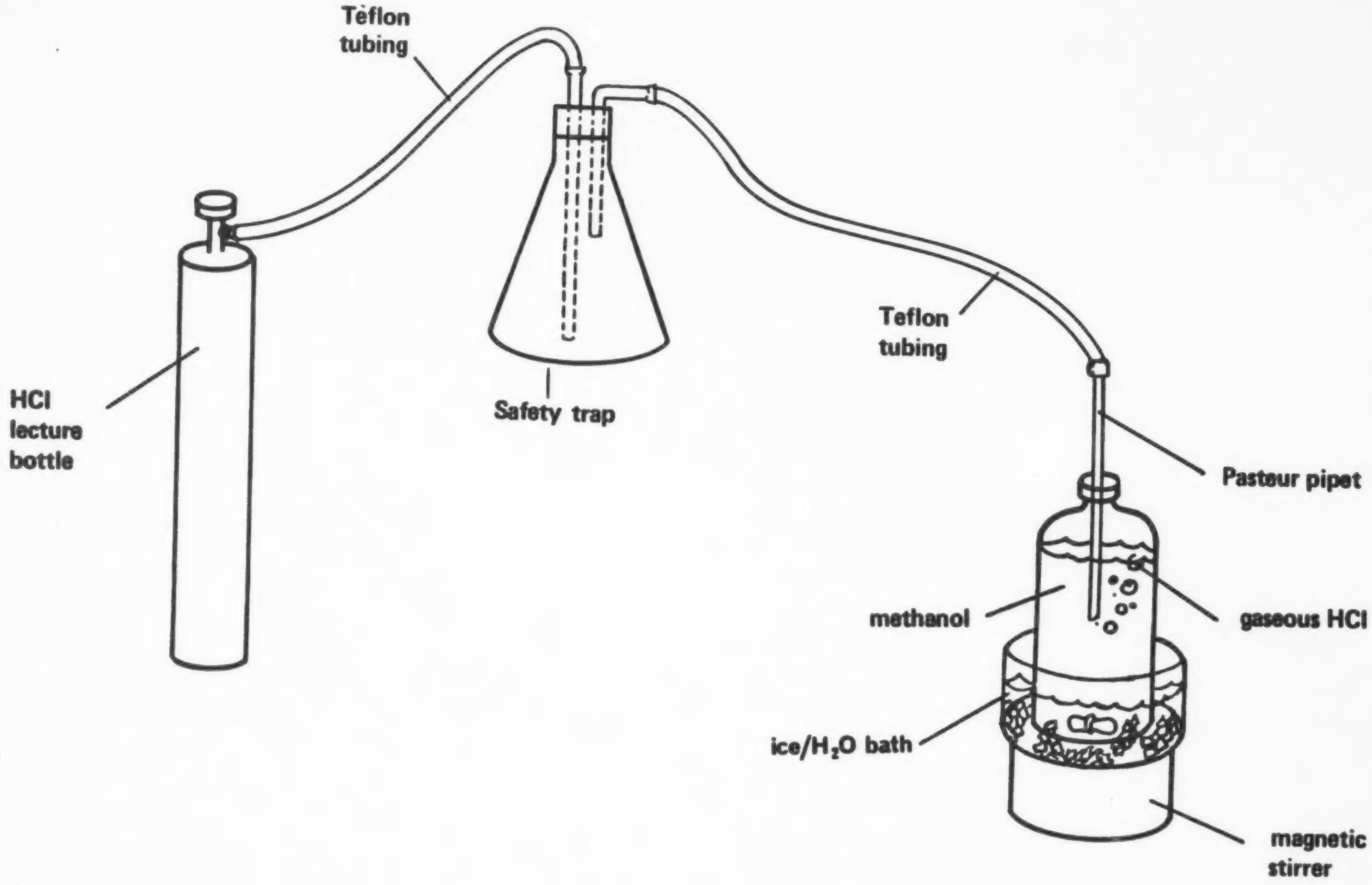

Teflon tubing
Safety trap
HCl lecture bottle
Teflon tubing
Pasteur pipet
methanol
gaseous HCl
ice/H_2O bath
magnetic stirrer

Figure 11.3. Equipment for generating HCl:methanol.

the methanol solution is determined by adding 1 mL of the methanol solution to 20 mL of deionized water, and titrating to the phenolphthalein endpoint with 1N KOH. A normality from 0.9 to 1.1 is acceptable for the analytical operation. If the solution is stored, the normality should be checked before use. Smaller amounts may be prepared in a similar manner.

11.3.5 Maximum Sample Size

Conductivity measurements must be determined for each sample to assure that the total concentration of anions in the sample does not exceed the resin capacity. Prior to measuring conductivity, check the pH of the sample using a pH meter. Adjust the sample pH to ~8 using 0.1N KOH or 0.1N H_2SO_4 if necessary. Table 11.3 lists the sample size which should be used for a given conductivity regardless of water type. Corresponding detection limits are also included.

11.3.6 GC/FID Performance Evaluation

Prior to analyzing procedural blanks, acceptable performance for the GC/FID system must be demonstrated.

(1) Analyze the GC/MS system performance standard as specified in Table 11.4. Figure 11.4 shows a total ion chromatogram of this mixture analyzed by GC/MS under similar conditions, which may be used to identify test components in the standard.

Table 11.3. ACCEPTABLE SAMPLE SIZE AND CORRESPONDING DETECTION LIMITS FOR SAMPLE CONDUCTIVITY RANGES

Conductivity Range (ohm)	Sample Size (mL)	Detection Limit (ppb)
<150	2500	1.5
150-300	1000	5
300-600	500	10
600-1,200	250	40
1,200-3,000	100	50
3,000-6,000	50	100
6,000-12,000	25	200
12,000-30,000	10	500
30,000-60,000	5	1000

Table 11.4. GC/FID OPERATING CONDITIONS FOR NONVOLATILE ACIDS

GC Column	30 m DB-5 (1.0 μ film thickness) fused silica, wide bore (0.34 mm i.d.), capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through column; 15:1 split injection
Temperature Program	35 to 180°C @ 5°/min
Injector Temperature	250°C
Detector Temperature	260°C
Injection Volume	1.0 μL

(2) Measure peak asymmetry (tailing) for acetophenone, 1-tetradecanol, and methyl d_7-naphthalene sulfonate using the percent peak asymmetry factor (PAF):

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

where

B = the width of the back half of a chromatographic peak measured at 10% above baseline

F = width of the front half of the chromatographic peak measured at 10% above baseline

PAF should measure less than 200% for acetophenone, 1-tetradecanol, and methyl d_7-naphthalene sulfonate.

(3) Check the acidity/basicity of the column by the peak area ratios determined by integrator or triangulation of 2,6-dimethylphenol to acetophenone. A ratio of 0.7 to 1.3 for both is acceptable.

(4) Sensitivity is checked by measuring signal-to-noise ratio for the methyl ester of 2,4,5-trichlorophenoxy acetic acid. A signal-to-noise ratio of 10 to 1 is acceptable.

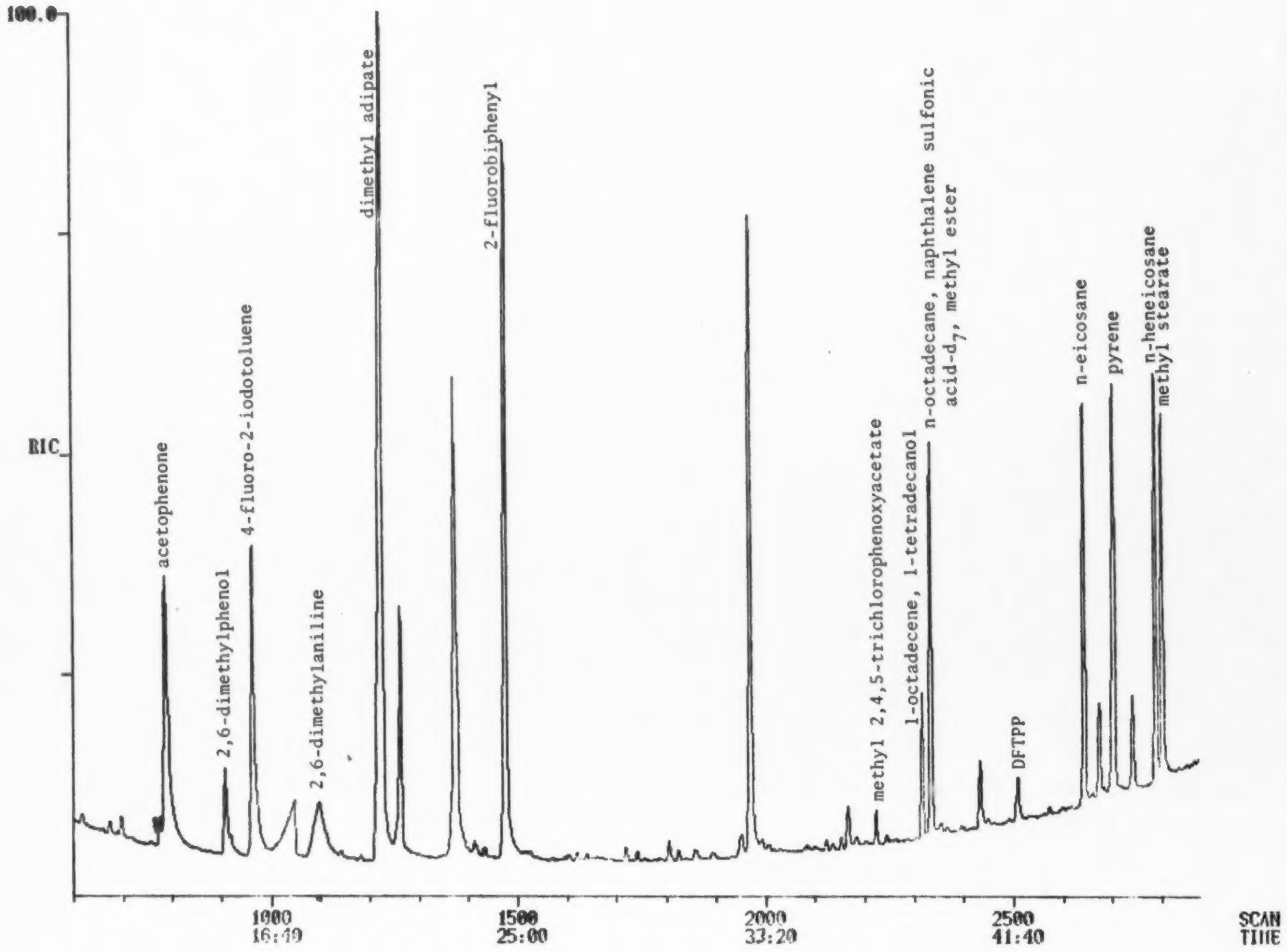


Figure 11.4. Total ion chromatogram for nonvolatile acids GC/MS performance standard.

11.3.7 Derivatization and Procedural Blanks and Controls

Prior to processing any samples a derivatization blank and a derivatization control must be run and analyzed by GC/FID to assure that background contamination is low and that derivatization yield is acceptable. After validating the derivatization step, three procedural blanks must be processed and analyzed by GC/FID to detect contamination and artifacts from the resin material, solvents, reagents, dirty glassware, and other sources.

11.3.7.1 Derivatization Blank--

(1) Add 0.2 mL of 1N HCl:methanol solution to a 15 mL round bottom test tube. Derivatize as described in steps 1 to 10, Section 11.4.2.

(2) With a 50 µL syringe, add 10 µL of the GC external standard (hexadecane) solution to the derivatized solution.

(3) Analyze by GC/FID using the conditions described in Table 11.4. Contaminant peak heights should be less the 20% relative to the peak height of the external standard (hexadecane).

(4) If significant contamination is present, repeat the derivatization blank using fresh glassware and reagents. Reagents and solvents may be distilled or taken from another source.

11.3.7.2 Derivatization Control--

(1) Prepare a control solution. With a 50 µL syringe, measure 25 µL of each stock solution in group B (Table 11.2) into a 10 mL volumetric flask and dilute to volume using pesticide grade acetone. Transfer into a Teflon sealed screw cap bottle and store at 4°C until use.

(2) With a 1 mL graduated pipette, measure 0.5 mL of this solution into a 15 mL round bottom test tube. Evaporate to ~0.1 mL using nitrogen blowdown at 40°C. Resuspend the sample in 0.2 mL of 1N HCl:methanol solution.

(3) Derivatize the sample using diazomethane as described in steps 1 to 10, Section 11.4.2.

(4) With a 50 µL syringe, add 10 µL of the GC external standard to the derivative mixture.

(5) Analyze samples by GC/FID as described in Table 11.4. Peaks heights of the control acids relative to the external standard should fall within the values given in Table 11.5.

(6) If derivative yield is low (as measured by relative peak heights) this step must be repeated using fresh reagents and glassware.

11.3.7.3 Procedural Blanks--

If the derivative blank and derivative control sample are acceptable, then a procedural blank must be run.

For each batch of materials and reagents that are used, a set of three procedural blanks are required. Table 11.6 identifies these blanks and defines their purpose. These blanks must be processed and analyzed by GC/FID prior to processing any samples. If a large number of samples are to be processed, it is advantageous to prepare large batches of materials and reagents thereby reducing the number of blanks which must be run. Procedural blank 1 must be run every time a set of samples is processed and analyzed.

11.3.7.3.1 Procedural Blank 1--

(1) Prepare a resin column as described in step 11.4.1.1.

(2) Follow the procedure for nonvolatile organic acid analysis as described in steps 11.4.1.5 to 11.4.2.10.

(3) With a 50 μL syringe, add 10 μI of the GC external standard to the derivatized mixture.

Table 11.5. RELATIVE PEAK HEIGHTS[a] (RPH) OF CONTROL ACIDS

Acid	Acceptable RPH Range
d_7-naphthalene sulfonic acid, methyl ester	.15-.20
adipic acid, dimethyl ester	.50-.60
2,4,5-trichlorophenoxyacetic acid, methyl ester	.50-.60

[a] $RPH = \frac{\text{peak height of derivatized acid}}{\text{peak height of external standard}}$

Table 11.6. PROCEDURAL BLANKS

Blank	Description
Procedural Blank 1	Detects contamination in resin material, solvents, and glassware
Procedural Blank 2	Detects contamination in reagent water
Procedural Blank 3	Detects contamination in sulfuric acid, sodium hydroxide, and sodium thiosulfate solutions used during sample collection and analysis

(4) Analyze samples by GC/FID as described in Table 11.4. Contaminant peak heights should measure less than 20% relative to the external standard.

(5) If significant contamination is present, the procedure should be repeated using a blank chromatography column with no resin added. If this blank is acceptable, then the resin is the source of contamination and should be recleaned using the procedure in Section 11.3.2. A procedural blank 1 should then be repeated using the fresh resin material.

(6) If significant contamination is present in this blank, fresh reagents and recleaned glassware should be used and the reagent blank repeated. When the reagent blank is acceptable, a procedural blank 1 must be repeated.

(7) Procedural blanks 2 and 3 may be processed once procedural blank 1 is acceptable.

11.3.7.3.2 Procedural Blanks 2 and 3--

(1) Prepare a resin column as described in step 11.4.1. Attach a separatory funnel to the top of the column. For Procedural blank 2, pour 100 mL of reagent water into the separatory funnel, then drain the sample through the resin bed at a flow rate of ~10 mL/min. For procedural blank 3 use 100 mL of reagent water spiked with 1 mL each of the sulfuric acid, sodium hydroxide and sodium thiosulfate solutions. These must be the same solutions

which are to be used when the samples are collected (Chapter 5, section 5.2.14).

(2) Follow the extraction procedure as described in steps 11.4.1(5) to 11.4.2(10).

(3) With a 50 μL syringe, add 10 μL of the GC external standard to each derivative mixture.

(4) Analyze the samples by GC/FID using the conditions in Table 11.4. Peak heights for contaminants in procedural blank 3 which are not present in procedural blank 2 should measure less than 20% relative to the external standard.

(5) If significant contamination is present in procedural blank 3 which is not present in procedural blank 2, the procedure should be repeated using fresh solutions of sulfuric acid, sodium hydroxide, and sodium thiosulfate, until an acceptable blank is achieved.

11.4 ION EXCHANGE SEPARATION AND DERIVATIZATION

11.4.1 Ion-Exchange Separation

(1) Prepare ion-exchange columns by placing a small glass wool plug in the bottom of the chromatography columns. Using a graduated pipette with the tip cut off, pipette 10 mL (measure volume after resin has settled) of cleaned Biorad AG 1-X8 resin into the open tubular chromatography columns, allow the resin to settle, and rinse the resin bed with ~10 mL reagent water.

(2) If the sample is turbid after pH adjustment/conductivity measurement, allow it to stand undisturbed for 4 hours or overnight prior to analysis to allow particulates to settle.

(3) Gently pour the aqueous portion of the sample (equilibrated to room temperature) into a graduated cylinder to measure sample volume. Both total volume of sample collected and the volume of sample analyzed should be recorded.

(4) For surface waters and effluents, transfer sample water to reservoirs on top of the column and allow it to drain through the resin bed. Flow is gravity controlled and should be approximately 5 mL/min (NOTE: The glass wool plug can restrict flow if packed too tightly). For drinking water, transfer sample to a

one gallon container. Attach siphoning adaptor between sample container and column. Attach a piece of tygon tubing between the end of the chromatography column and a water aspirator. To start sample flow through the resin bed, open stopcock on the chromatography column and turn on aspirator. This should create sufficient suction to pull the water sample through the siphon adaptor and onto the resin column. Disconnect suction after water is flowing through the resin bed. Using the stopcock at the base of the resin bed, adjust flow to 2.5 mL/minute for a 2500 mL sample volume. At this flow rate, it will require ~16 hours for the sample to drain through the column, which is suitable for overnight accumulation. Allow the water to drain out of the resin bed. If particulate matter in the sample plugs the column, positive head pressure can be used to restore flow.

(5) Rinse the resin with 25 mL deionized water. Allow the water to drain out of the resin bed, aspirate off remaining water from the bottom of the resin column. Rinse the resin with 100 mL methanol. First, pass a fraction of the methanol (~50 mL) through the column, stop solvent flow, remove the reservoir, stopper the column with a ground glass stopper and shake the column to remove air bubbles. Pass remaining methanol through the resin. Stop flow when methanol reaches the top of the resin column. Rinse at ~5 mL/minute, discard rinse.

(6) Elute acids from the column using 100 mL of 1N HCl:methanol. Collect the eluant in a 200 mL round bottom flask. Elute at ~5 mL/minute.

(7) Remove all of the eluant solvent, except any water residual using rotary evaporation at 30°C. Residual water is removed by adding 50 mL of methyl-t-butyl ether to the flask and evaporating at ~40°C. If any water still remains, add a second 50 mL portion of methyl-t-butyl ether and repeat the concentration step. Evaporation should require 15 to 20 minutes per sample (NOTE: sample must evaporate to dryness to remove HCl).

(8) Resuspend the sample residue in 3 mL of methanol and transfer to a 15 mL test tube. Rinse the flask with two 2 mL portions of

methanol and transfer to the test tube. Flasks must be rinsed thoroughly to avoid loss of sample components.

(9) Evaporate the solvent to dryness using nitrogen blowdown at 40°C.

(10) Resuspend the sample in 0.2 mL of 1N HCl:methanol solution. Sample may be stored at this point.

(11) Derivatize using diazomethane.

11.4.2 Derivatization

CAUTION: Diazomethane is toxic and prone to cause development of specific sensitivity; in addition, it is potentially explosive. Hence one should wear heavy gloves and goggles while performing this experiment and should work behind a safety screen or a hood door with safety glass. Also, it is recommended that ground joints and sharp surfaces be avoided. Thus all glass tubes should be carefully fire-polished, connections should be made with rubber stoppers or clear glass seals, and separatory funnels should be avoided, as should etched or scratched flasks. Furthermore, at least one explosion of diazomethane has been observed at the moment crystals (sharp edges!) suddenly separated from a supersaturated solution. Stirring by means of a Teflon coated magnetic stirrer is preferred to swirling the reaction mixture by hand, for there has been at least one case of a chemist whose hand was injured by an explosion during the preparation of diazomethane in a hand-swirled reaction vessel.

It is imperative that diazomethane solutions not be exposed to direct sunlight or placed near a strong artificial light, because light is thought to have been responsible for some of the explosions that have been encountered with diazomethane. Particular caution should be exercised when an organic solvent boiling higher than ether is used. Because such a solvent has a lower vapor pressure than ether, the concentration of diazomethane in the vapor above the reaction mixture is greater and an explosion is more apt to occur.

Most diazomethane explosions take place during its distillation. When distilled diazomethane is required, the present procedure is particularly good because at no time is much diazomethane present in the distilling flask.

(1) Assemble the diazomethane generation/distillation apparatus (Figure 11.5).

(2) Place 10 mL fresh 60% KOH (w/v) in the distilling flask with 35 mL ethanol and 10 mL of diethyl ether (CAUTION: flammable; forms explosive peroxides). Add a magnetic stirring bar.

(3) Place ice/salt mixture in the condenser and place the collection flask in a ice/salt bath.

(4) Prepare a solution of 21.5 g (0.1 mole) of p-tolylsulfonylmethylnitrosamide (Daizald®, Aldrich) in 125 mL of ether. Larger amounts of ether may be needed to dissolve the reagent if it is especially pure or the temperature is less than 20°C. Place the Diazald® reagent in the dropping funnel.

(5) The distilling flask is heated in a water bath at 70-75°C, the stirrer is started.

(6) The Diazald® solution is added at a regular rate over a 15-20 minute period. As soon as this solution has been added, add 100 mL of ether to the dropping funnel and continue to add the ether at the same rate used for the reagent.

(7) Continue distillation until the distillate is colorless.

(8) Cool the distillation flask. Remove the collection flask and keep cold until used. Destroy any residues of diazomethane in the apparatus and distilling flask by rinsing with 1% formic acid in ether.

(9) With a Pasteur pipette add the distilled diazomethane-ether solution to the sample extracts until a yellow color persists. For highly colored samples add the diazomethane solution until nitrogen evolution ceases. Additional diazomethane should be prepared as needed. Excess diazomethane is evaporated from samples during nitrogen blowdown. If excess diazomethane reagent has been prepared it should be quenched with formic acid solution (1% in ether) before discarding. The solution will be colorless when fully quenched.

(10) Concentrate the derivatized samples to 0.5 mL in the test tubes using nitrogen blowdown at ~30°C. Since excess diazomethane is

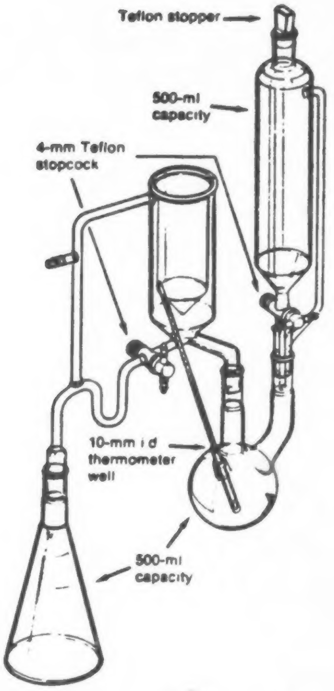

Teflon stopper
500-ml capacity
4-mm Teflon stopcock
10-mm i d thermometer well
500-ml capacity

Figure 11.5. Z10,851-0 Macro Diazald® set (with ₮ Clear-Seal® joints).

still present in the samples this step must be performed in a well ventilated hood.

(11) Store the samples at 0°C until analysis.

(12) With a 50 μL syringe, add a 10 μL aliquot of the MS external standard solution to the sample concentrate immediately before analysis.

11.5 GC/MS/COMP ANALYSIS

Prior to beginning GC/MS/COMP analysis of the nonvolatile acids (NOVA), the analyst should be familiar with procedures described in Chapter 13.

11.5.1 Preparation for Analysis

11.5.1.1 GC/MS/COMP Operating Parameters--

The recommended GC/MS operating parameters for the analysis of NOVA fractions are given in Table 11.7. (This capillary column should not be used for the analysis of any fractions from other protocols.)

Table 11.7. GC/MS OPERATING CONDITIONS FOR NONVOLATILE ACIDS AS THEIR METHYL ESTERS

GC Column	30 m DB-5 (1.0 μ film thickness) fused silica wide bore (0.34 mm) capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through column; 9:1 split injection
Carrier Gas Sweep Time	85 sec (50°C)
Temperature Program	50°C/5 min; to 250°C @ 4°/min and hold
Injector Temperature	250°C
Transfer Line Temperature	255°C
Injection Mode	Splitless, 0.4 min/split
Injection Volume	1.0 μL
Ionizing Energy	70 eV
Ion Source Temperature	250°C
Scan Range	35-500
Scan Rate	2 sec (1.9 sec scan, 0.1 sec settling)

11.5.1.2 MS Calibration--

The mass spectrometer is calibrated using the manufacturer's recommended approach (Chapter 13). The acceptability of the calibration results is verified by the analysis of the NOVA system performance solution (SPS).

11.5.2 Analysis of SPS (Quality Control)

Using the above prescribed GC/MS conditions the SPS (Table 11.1) is analyzed and all necessary data is acquired and evaluated prior to proceeding to sample analysis. The SPS is analyzed at the beginning of each day's operation.

The SPS raw data is extracted from the run, and test parameters calculated and plotted or tabulated (Tables 11.8 and 11.9). Up to 14 days of SPS analytical results can be historically recorded in Table 11.8 for day-to-day comparisons. This allows the analyst to follow subtle trends that may develop in anticipation of GC or MS maintenance requirements. Chapter 13 explains calculation and usage of these data.

Table 11.8. GC-MS SYSTEM PERFORMANCE TEST FOR NONVOLATILE ACIDS (NOVA)

Dates: ________ ________

Run Id Code ________ ________

GC Column and Program: ____

Notebook References: ____

MS SYSTEM CHECKS

High/Low Mass Balance

DFTPP

Ion	Relative Abundance Criteria
51	30% to 60% of m/z 198
68	<2% of m/z 69
70	<2% of m/z 69
127	40% to 60% of m/z 198
197	<1% of m/z 198
198	100%
199	5-9% of m/z 198
275	10% to 30% of m/z 198
365	at least 1% of m/z 198
441	present, but < m/z 443
442	>40% of m/z 198
443	17% to 23% of m/z 442

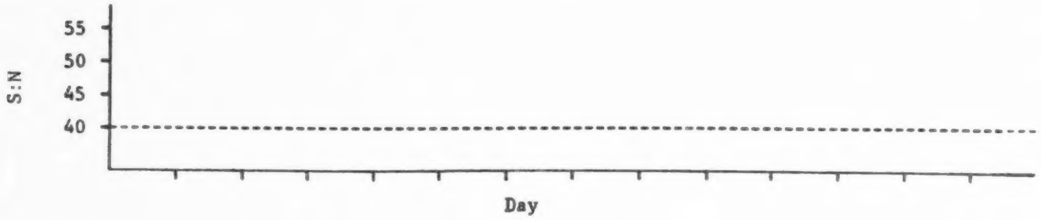
m/z	51	68	70	127	197	198	199	275	365	331	442	443	Pass/ Fail	Remedial Action
Abundance * Criteria	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )		
Day														

*These abundances are initially established by the MAS user, and subsequently become guidelines for acceptability of tune.

(continued)

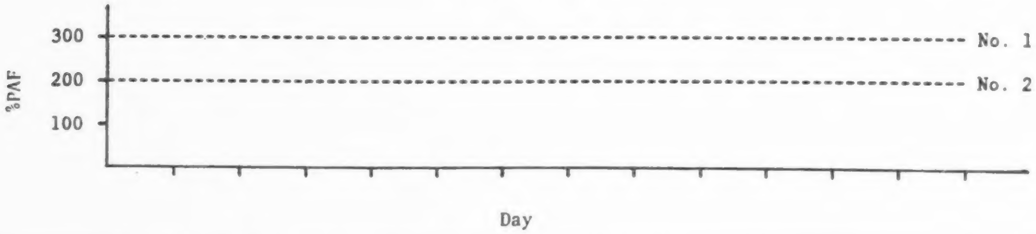
Table 11.8 (cont'd.)

Test	Components	Criteria
Limit of Detection	Methyl 2,4,5-Trichloro phenoxy Acetate	S:N (m/z 73) > 3:1

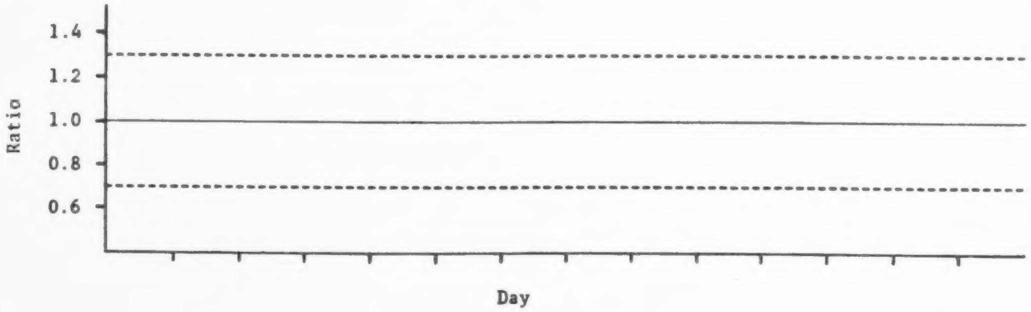


GC SYSTEM CHECKS

Test	Components	Criteria
Peak Asymmetry	1. Acetophenone (TIC) ● 2. 1-Tetradecanol (TIC) ■	%PAF <300 %PAF <200



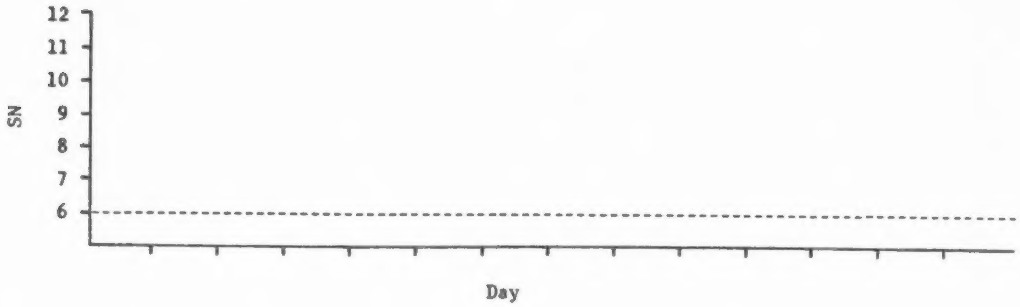
Test	Components	Criteria
Basicity	1. Acetophenone (TIC) 2. 2,6-Dimethylphenol (TIC)	Ratio 2:1 = 0.7 to 1.3

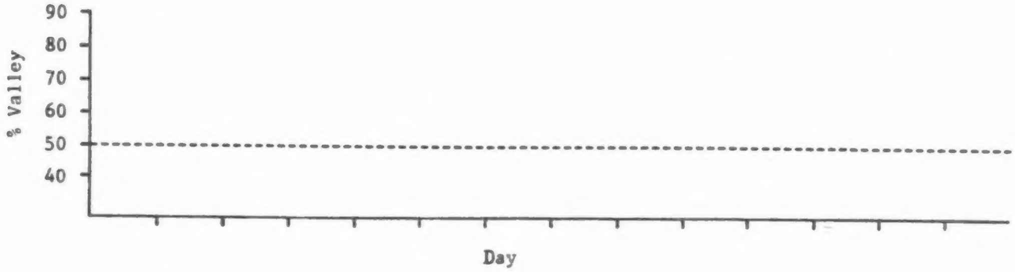


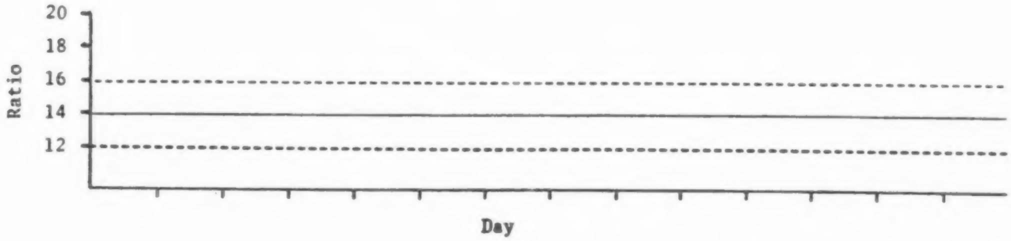
(continued)

Table 11.8 (cont'd.)

Test	Components	Criteria
Separation Number	n-Eicosane (m/z 43) n-Heneicosane (m/z 43)	SN ≥ 6
Resolution	n-Octadecane (TIC) 1-Octadecene (TIC)	R = minimum 50% Valley
Inertness	Methyl Stearate	Ratio m/x 74 to 298 = 12:1 to 16:1



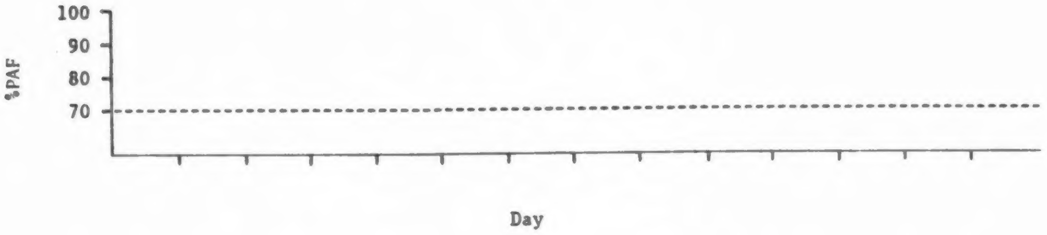




(continued)

Table 11.8 (cont'd.)

Test	Components	Criteria
Capillary Capacity	Dimethyl Adipate (TIC)	%PAF >70



Test	Components	Criteria
Relative Retention Shift	1. Heneicosane 2. Pyrene	RRS = 0.95-1.05

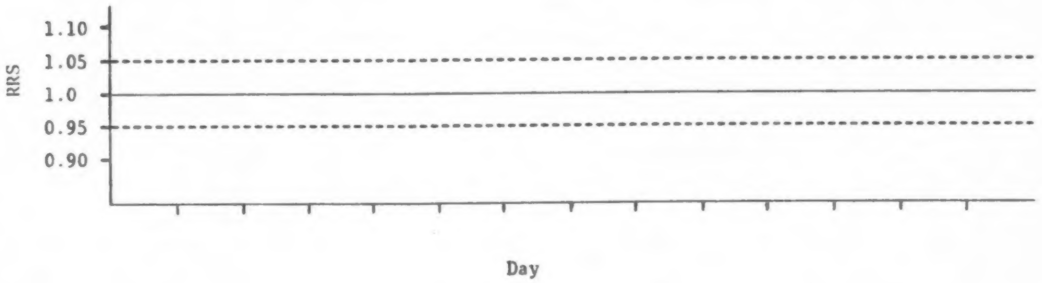


Table 11.9. GC-MS SYSTEM PERFORMANCE TEST: RMR CHECK FOR NONVOLATILE ACIDS (NOVA)

Date: ____________________ Run ID Code: ____________________

DATA

Standard	MW	Amount (ng)	μM	Ion (m/z)	Area (Run 1)	Area (Run 2)
2-Fluorobiphenyl	172			172		
4-Fluoro-2-Iodo-toluene (FIT)	236			109		
				236		
d_7-Naphthalene sulfonic acid	229			134		
				229		

MATRIX OF STANDARD ION RMRs

Standard	Ion	172	109	236	134	229
2-Fluorobiphenyl	172	-				
FIT	109		-			
	236			-		
d_7-Naphthalene sulfonic acid	134				-	
	229					

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

Additional QC/QA procedures include a check on the RMRs on a daily basis (Table 11.9). These RMR data are compared to those obtained during the development of the historical data bank. The usage of these data are also discussed in Chapter 13.

11.5.3 Determination of RMRs

RMRs for the User's data bank (as opposed to the daily RMR checks discussed in the above paragraph) are determined by injecting a solution containing the derivatized internal standards and analytes.

The GC/MS/COMP operating parameters are given in Table 11.7. Table 13.8 may be used to tabulate RMR raw data.

11.5.4 Analysis of Field and Quality Control Samples

After all specified criteria for GC and MS performance (Tables 11.8 and 11.9) have been met, analysis of standards, QA/QC, or NOVA sample extracts is conducted.

Qualitative and quantitative procedures are described in Chapter 13. Table 11.10 may be used to tabulate RMR and sample analyte raw data, prior to using the MASQUANT software program or manual quantitative calculation.

Table 11.10. RAW ANALYTE DATA FOR NOVA FRACTION

Date: ____________ Run I.D. Code: ____________

Sample Identification: ______ Notebook Reference: ________

Vol. Water (L): __________ Vol. Water Processed (mL): ____
(to which is were added)

STANDARDS

	I.D. No.	Spectrum No.	Weight (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
2-Fluorobiphenyl				172:	___, ___	___	___, ___
4-Fluoro-2-Iodotoluene				109,236:	___, ___	___	___, ___
d_7-Naphthalene sulfonic acid				134,229:	___, ___	___	___, ___

ANALYTES

Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Area	MW_x^a
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	
			___, ___:	___, ___	

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{RMR_{x/y}} \times \frac{ng_y}{A_y \cdot MW_y}$$

$$K = \frac{ng_y}{A_y \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

CHAPTER 12

CATION-EXCHANGE AND ANALYSIS OF STRONG AMINES (SAM)

12.1 INTRODUCTION

12.1.1 Principle of the Technique

This technique describes the determination of volatile primary, secondary, and tertiary amines in drinking water, surface waters, and treated wastewater effluents. Although some amines can be extracted from water with organic solvents, ion-exchange concentration offers several important advantages. First, the recovery of primary amines and low molecular weight secondary amines may be low during solvent extraction. Second, samples tend to emulsify at alkaline pH during solvent extraction. Finally, larger volumes of water can be processed when utilizing accumulator ion-exchange columns thereby giving lower detection limits.

This procedure utilizes cation-exchange resins to concentrate amine salts from the aqueous matrix. Amines are eluted from the resin material using NaOH in an acetonitrile:water solution. The base deprotonates the amine cation and the free amines are then rinsed off the resin with the acetonitrile:water solution. The amines are then converted to their nonvolatile HCl salts and the solution is evaporated to dryness. The residue is resuspended in 3 mL water, and sufficient NaOH is added to give a pH greater than 13, which converts the amines to their free base form. Amines are then extracted into 6 mL of methyl- t-butyl ether. Amines should be derivatized prior to GC/MS analysis to prevent adsorptive losses during chromatography. Primary amines are derivatized with pentafluorobenzaldehyde (SAM-PT fraction). Secondary amines are derivatized with pentafluorobenzylbromide (SAM-S fraction). Since the volatile amines have been converted to less volatile derivatives, derivatized samples may be concentrated using nitrogen blowdown prior to analysis. Because the primary and secondary amines are not derivatized with the same derivatizing reagent, the sample must be split prior to derivatization. If the conductivity of the sample extract water is low, a larger sample volume may be used and the sample split immediately before derivatization. However, for samples with high conductivity, the sample must be split prior to ion-exchange concentration to achieve the specified detection limits.

Tertiary amines are also separated by this protocol and quantified (underivatized) in the primary amine fraction.

12.1.2 Detection Limits

If an average detection limit for GC/MS analysis is estimated as 10 ng for each sample component for derivatized amines, and the final volume is 0.3 mL, then nominal detection limits for the various water types using a 1 μL injection are directly related to sample size. Detection limits for a sample (split before ion-exchange concentration) are:

1 ppb for a 2.5 L sample (drinking water);
5 ppb for a 500 mL sample (surface water);
10 ppb for a 250 mL sample (industrial and municipal wastewater effluents); and
50 ppb for a 50 mL sample (energy effluents).

12.1.3 Interferences

By-products from the derivatizing reagent can cause interferences during the analysis of secondary amines if excess KOH is present during derivatization. The primary amines are derivatized with a different reagent which does not form chromatographable by-products. A procedural blank must be run prior to processing any samples to assure that contamination from resin material, solvents, reagents, glassware, and other sources is low.

12.1.4 Precision, Accuracy, and Scope

Table 1.9 in Chapter 1 presents average recoveries and standard deviations for a variety of primary, secondary, and tertiary amines. The procedure gives acceptable recoveries for low molecular weight aliphatic amines. Piperidine and morpholine do not give acceptable recoveries. Certain weak bases may be detected in these fractions, but are measured in the pH 8 extractable fraction (WABN) where they are extracted more efficiently.

12.2 APPARATUS AND REAGENTS

The following materials are required for processing a set of nine samples plus three procedural blanks. Nine is the maximum number of samples (field and quality control) which should be processed at a time. The quality control samples that should be run during these analyses are listed in Table 5.1, Chapter 5. The three procedural blanks must be run before any samples are collected or processed. When the samples are split after concentration, nine samples can be processed for both primary and secondary amines. When the samples are split prior to ion-exchange concentration, the nine samples can be processed for either primary or secondary amines. It will require two to three days to process nine samples, depending upon the sample size.

(1) Nine glass chromatography columns, ~1 cm I.D. × 22 cm with 500 mL sample reservoir (Figure 12.1). The size of the sample reservoir is not critical; however, a 500 mL reservoir will hold the entire sample for all water types except drinking water. For drinking water an adaptor (Figure 12.2) for siphoning the sample onto the resin should be used in lieu of a sample reservoir. This allows unattended flow through the sample for a 14 to 16 hr period such that the sample can be concentrated overnight. A vacuum aspirator is needed to start sample flow through the siphoning adaptor and resin column.

(2) One 10 mL graduated pipette with the tip cut off for measuring and transferring resin material.

(3) One graduated cylinder for measuring sample volume. The size of the sample, thus, the cylinder will depend upon the results of conductivity measurements. In general it may be:

1000 mL for drinking water
500 mL for surface water
250 mL for industrial and municipal wastewater effluents
50 mL for energy wastewater effluents

The cylinder may be reused for each sample if it is rinsed 5 times with distilled-deionized water.

(4) One conductivity bridge - range 1 to 30,000 ohm.

(5) Nine 200 mL round bottom flasks (24/40).

(6) One rotary evaporator. If more than one is available up to four samples can be evaporated at a time. Samples may bump initially

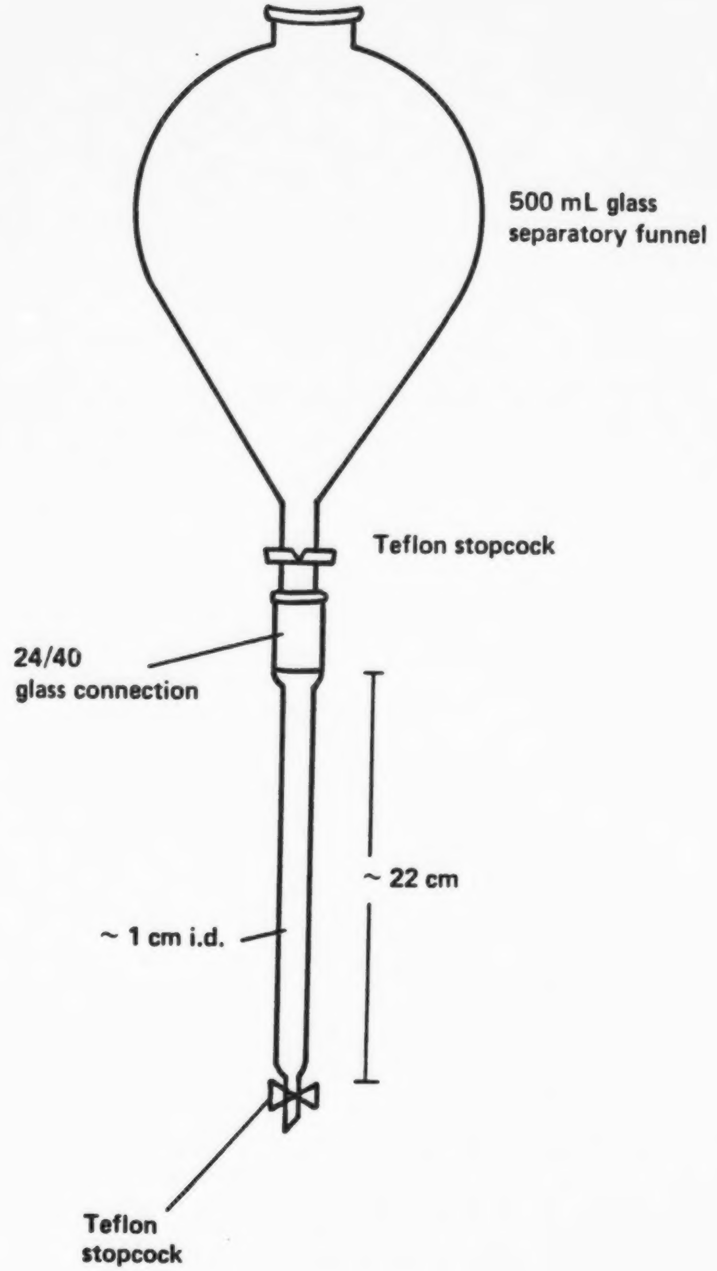

500 mL glass
separatory funnel
Teflon stopcock
24/40
glass connection
~ 22 cm
~ 1 cm i.d.
Teflon
stopcock

Figure 12.1. Chromatography column with sample reservoir (exact dimensions of column are not critical - must hold 10 mL of resin).

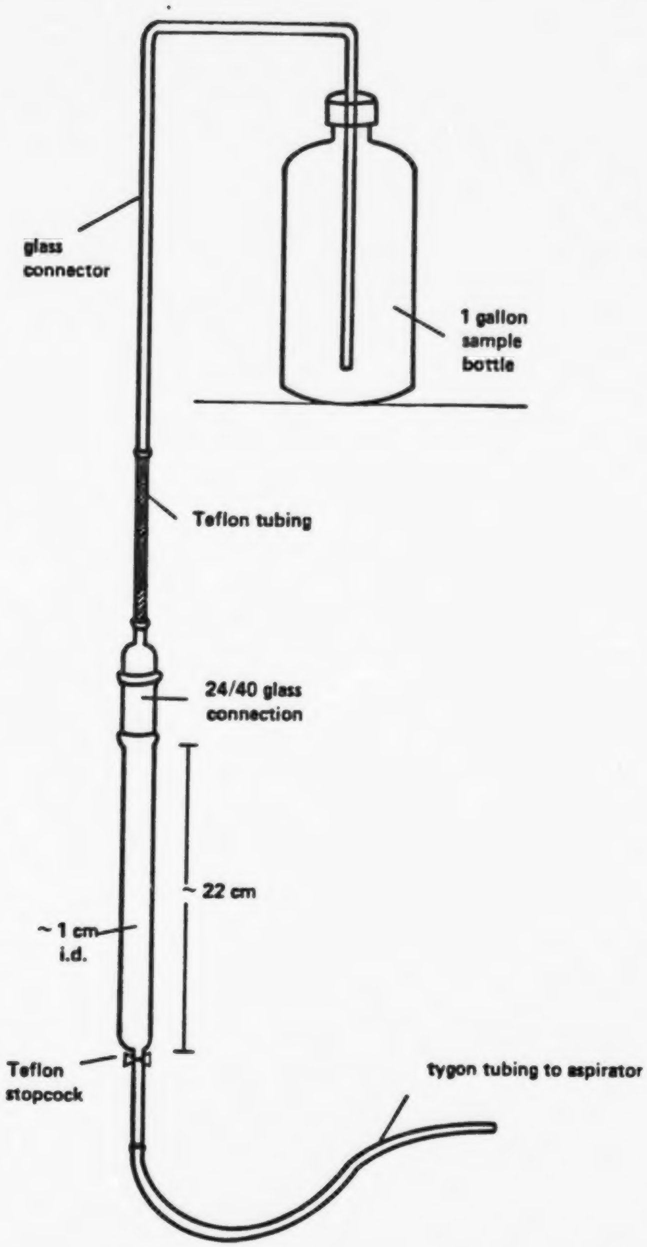

glass connector
1 gallon sample bottle
Teflon tubing
24/40 glass connection
~ 22 cm
~ 1 cm i.d.
Teflon stopcock
tygon tubing to aspirator

Figure 12.2. Chromatography column with siphoning adaptor for drinking water.

but after acetonitrile has evaporated the samples require little attention.

(7) Small (15 mL) round bottom test tubes with 15 mm screw caps (Supelco 3-3112) and Teflon lined rubber septa (Supelco 3-3115). Specified materials must be used to prevent solvent evaporation during derivatization. Sixty tubes (2 for each sample) are required if the sample is split after concentration. Thirty tubes (1 for each sample) are required if the sample is split prior to ion-exchange concentration. An additional twenty test tubes will be needed for processing primary amines.

(8) One Soxhlet extractor for cleaning ion-exchange resin. A minimum 150 mL capacity is required. However, resins can be processed in large batches and stored; therefore a 500 mL or 1000 mL size would be preferred.

(9) One large chromatography column for preparing resin material. The dimensions of this column are not critical, it should hold a minimum of 160 mL of resin material; for large batches of resin a larger column is preferred. The flow through the column should not exceed 50 mL/minute.

(10) 10 mL volumetric flasks for preparing standard solutions.

(11) 15 mL vials with Teflon lined screw caps for storing standard solutions.

(12) Syringes for preparing standard solutions - 50 and 150 µL.

(13) Graduated pipettes for preparing standard solutions - 1 mL.

(14) One heating block capable of maintaining 60°C with at least ten spaces for 15 mL round bottom tubes.

(15) One manifold with a temperature controlled water bath or heating block for nitrogen blowdown. A manifold with nine to eighteen spaces is preferred but not required.

(16) One vortex mixer - vortex Genie, Fisher Scientific or equivalent.

(17) One clinical centrifuge capable of ~4000 rpm.

(18) One pH meter with a combination electrode.

(19) One gas chromatograph suitable for capillary column chromatography and all required accessories including syringes, gases, flame ionization detector and a strip chart recorder.

(20) One 30 m × 0.34 mm I.D. DB-1 (1.0 μ film thickness) fused silica capillary column.

(21) Materials and Reagents

- (a) 160 mL AG 50 W-X8 (50-100 mesh) cation-exchange resin (Biorad Laboratories, Richmond, CA) in the H^+ form.
- (b) Reagent water - reagent water is described as a water source that does not produce a background interference at the limit of detection and which has a conductivity of <1 ohm. A water purification system (Millipore Super Q or equivalent) may be used to generate reagent water.
- (c) Methanol (pesticide analysis grade).
- (d) 1 L 0.57N NaOH in acetonitrile:reagent water (10:25).
- (e) 200 mL concentrated HCl.
- (f) 10 mL 10N NaOH in reagent water.
- (g) 200 mL 0.1N NaOH in reagent water (use the same solution as specified in Chapter 5, section 5.2.14).
- (h) 200 mL 0.1N H_2SO_4 in reagent water (use the same solution as specified in Chapter 5, section 5.2.14).
- (i) 200 mL 0.05M $Na_2S_2O_3$ in reagent water (use the same solution as specified in Chapter 5, section 5.2.14).
- (j) 100 mL methyl-t-butyl ether (pesticide analysis grade).
- (k) Reagents for derivatizing primary amines.
  - 50 mL pentafluorobenzaldehyde in methanol (2.5 g/10 mL). Recently purchased reagent does not require distillation before use.
- (l) Reagents for derivatizing secondary amines.
  - 50 mL pentafluorobenzylbromide in acetone (5% w/v). Recently purchased reagent does not require distillation before use. (Available from Aldrich as α-bromo-2,3,4,5,6-pentafluorotoluene).
  - 1 mL triethylamine.
- (m) 1 mL MS external standard solution - 4-fluoro-2-iodotoluene (300 ng/10 μL) and 2-fluorobiphenyl (300 ng/10 μL) in CH_2Cl_2.

(n) 1 mL GC external standard solution - hexadecane (30 ng/10 μL) in CH_2Cl_2.

(o) 1 mL system performance standard solution for the primary amines (Table 12.1) and the secondary amines (Table 12.2).

12.3 PREPARATION FOR ANALYSIS

12.3.1 Preparation of System Performance Solutions

(1) Prepare individual stock standards as specified in Table 12.3.

- solids - accurately weight 0.120 g of pure material, dissolve in the specified solvent, dilute to volume in a 10 mL volumetric flask.

Table 12.1. GC/MS SYSTEM PERFORMANCE SOLUTION FOR PRIMARY AND TERTIARY AMINES (SAM-PT)

Compounds	Concentration (ng/μL)
2,6-dimethylaniline	300
acetophenone	310
1-tetradecanol	240
1-octadecene	240
n-octadecane	230
DFTPP	300
n-eicosane	300
pyrene	300
n-heneicosane	300
methyl stearate	10
2-fluorobiphenyl	300
4-fluoro-2-iodotoluene	270
n-butylamine-d_9 (PFB Schiff[a] base)	290[b]
phenylethylamine-d_4 (PFB Schiff[a] base)	300[b]
allylamine (PFB Schiff[a] base)	16[b]
n-hexylamine (PFB Schiff[a] base)	990[b]

[a] Derivatized with pentafluorobenzaldehyde.

[b] Concentration of underivatized amine.

Table 12.2. GC/MS SYSTEM PERFORMANCE SOLUTION FOR SECONDARY AMINES (SAM-S)

Compounds	Concentration (ng/µL)
2,6-dimethylaniline	290
acetophenone	310
1-tetradecanol	240
1-octadecene	240
n-octadecane	230
DFTPP	300
n-eicosane	300
pyrene	300
n-heneicosane	300
methyl stearate	10
N-ethyl-2-fluorobenzylamine (PFB[a] derivative)	300[b,c]
2-fluorobiphenyl	300
4-fluoro-2-iodotoluene	270
diallylamine (PFB[a] derivative)	16[c]
morpholine (PFB[a] derivative)	990[c]

[a] Derivatized with pentafluorobenzylbromide.

[b] No adjustment made for density.

[c] Concentration of underivatized amine.

- liquids - with a 250 µL syringe, accurately measure 120 µL of pure liquid, dissolve in the specified solvent, dilute to volume in a 10 mL volumetric flask. To determine the quantity of N-ethyl-2-fluorobenzylamine added to the solution, weigh the dry syringe, then weigh the syringe containing the neat compound. The difference between these two weights is the weight of material delivered to the solution. The syringe must be rinsed well with solvent to assure that all of the neat material is delivered.

(2) Prepare a secondary, neutral standard. With a 1 mL graduated pipette, measure 0.5 mL of each stock standard in group A (for

Table 12.3. STOCK STANDARD SOLUTIONS

Compounds	Density (@ 20°C)	Concentration mg/10 mL
Standard A[a]		
2,6-dimethylaniline	0.984	120
acetophenone	1.030	120
1-tetradecanol	0.823	98
1-octadecene	0.789	95
n-octadecane	0.777	93
DFTPP	S	120
n-eicosane	S	120
pyrene	S	120
n-heneicosane	S	120
methyl stearate	S	120
2-fluorobiphenyl	S	120
4-fluoro-2-iodotoluene	0.883	120
Standard B[b]		
n-butylamine-d_7	0.740	89
allylamine	0.761	91
n-hexylamine	0.766	92
2-phenylethyl-1,1,2,2-d_4-amine	0.965	120
Standard C[b]		
diallylamine	0.787	94
morpholine	0.999	120
N-ethyl-2-fluorobenzylamine[c]	unknown	120[c]

[a] Solvent is methylene chloride.

[b] Solvent is methyl-*t*-butyl ether.

[c] No adjustment made for compound density.

methyl stearate measure 17 μL with a 50 μL syringe) into a 10 mL volumetric. Dilute to volume using pesticide grade methylene chloride. Transfer into a Teflon sealed screw cap bottle. Store at 4°C.

(3) Prepare a secondary standard for the primary amines. With a 1 mL graduated pipette, measure 0.65 mL of the n-butylamine, 0.5 mL of the phenylethylamine, and 2.15 mL of the hexylamine standards into a 10 mL volumetric flask. With a 50 μL syringe add 35 μL of the allylamine stock standard to the same flask. Dilute to volume using pesticide grade methyl-t-butyl ether. Transfer into a Teflon sealed screw cap bottle. Store at 4°C.

(4) Prepare a secondary standard for the secondary amines. With a 1 mL graduated pipette, measure 0.5 mL of the N-ethyl-2-fluorobenzylamine and 1.65 mL of the morpholine standards into a 10 mL volumetric flask. With a 50 μL syringe, add 35 μL of the diallylamine stock standard to the same flask. Dilute to volume using pesticide grade methyl-t-butyl ether. Transfer into a Teflon sealed screw cap bottle. Store at 4°C.

(5) Fresh standards should be prepared every six months. If degradation or evaporation has occurred, fresh standards should be prepared sooner.

(6) If compound purity is 96% or greater, the weight or volume can be used without correction to calculate the concentration of stock standards. Compounds which are less than 96% pure cannot be used for standards.

(7) For the primary amine SPS (Table 12.1), with a 1 mL graduated pipette, measure 0.5 mL of the primary amine secondary standard solution into a 15 mL round bottom test tube. Add 5 mL methyl-t-butyl ether. Derivatize and extract as described in step 15(a), section 12.4. Evaporate the sample to 0.5 mL, then with a 1 mL graduated pipette, add 0.5 mL of the secondary neutral standard solution. Cap the test tube. Store at 0°C until GC/MS analysis.

(8) For the secondary amine SPS (Table 12.2), with a 1 mL graduated pipette, measure 0.5 mL of the secondary amine secondary standard

solution into a 15 mL round bottom test tube. Add 5 mL methyl-t-butyl ether and derivatize as described in step 15(b), Section 12.4. Evaporate the derivatized sample to 0.5 mL, then with a 1 mL graduated pipette, add 0.5 mL of the secondary neutral standard solution. Cap the test tube. Store at 0°C until GC/MS analysis.

12.3.2 Preparation of Resin Material

Ion-exchange resin is cleaned prior to preparing the resin columns. One hundred and twenty mL of resin material is needed to process 9 samples and three procedural blanks and is the minimum amount of resin which should be prepared. The following instructions describe procedures based on 120 mL of resin material; however, it is possible to prepare larger batches and store the prepared resin. If a larger volume of resin is prepared, volumes for rinsing the resin should be adjusted accordingly.

Biorad AG 50W-X8 (50-100 mesh) in the H^+ form is extracted with redistilled pesticide analysis grade methanol in a Soxhlet extractor overnight. The resin is placed in a column and rinsed with 10 bed volumes (1.2 L) of deionized water. Resin which is not used immediately may be stored wet, sealed in glass jars.

12.3.3 Cleaning of Materials

(1) All glassware to be used is washed with Amway S-A-8 laundry compound (or isoclean or other nonionic detergent) rinsed with deionized water and baked for a minimum of 4 hours at 500 to 550°C. All cleaned glassware is immediately capped or covered with foil (precleaned with hexane) to prevent contamination.

(2) Teflon liners and teflon lined septa are sonicated for 10 minutes in pesticide grade methanol followed by 10 minutes in pesticide grade pentane. The sonicated liners are vacuum-oven dried for 3 to 5 hours at 70°, ~28 inches of water, and stored in clean, Teflon lined screw-capped bottles.

12.3.4 Maximum Sample Size

Conductivity measurements must be determined for each sample to assure that the total concentration of cations in the sample will not exceed the resin capacity. Prior to measuring conductivity, check the pH of the sample using a pH meter. Adjust the sample pH to 4 to 5 using 0.1N

KOH or 0.1N H_2SO_4. Because the primary and secondary amines are not derivatized under similar conditions, the sample must be split prior to derivatization. If the conductivity of the sample water is low, a larger sample volume may be used and the sample split immediately before derivatization. However, for samples with high conductivity, smaller volumes must be used. The sample is split prior to ion-exchange concentration to achieve the specified detection limits. Table 12.4 list the sample size which should be used for a given sample conductivity regardless of water type. Corresponding detection limits both with and without sample split prior to derivatization are given for each sample size.

12.3.5 GC/FID Performance Evaluation

Prior to analyzing procedural blanks, acceptable performance for the GC/FID syst must be demonstrated.

(1) Analyze the GC/MS system performance standards as specified in Table 12.5. Figures 12.3 and 12.4 show total ion chromatograms of both mixtures analyzed by GC/MS under similar conditions which may be used to identify test components in the standards.

(2) Measure peak asymmetry or tailing for acetophenone and 1-tetradecanol using the percent peak asymmetry factor (PAF):

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

where

B = the width of the back half of a chromatographic peak measured at 10% above baseline.

F = width of the front half of the chromatographic peak measured at 10% above baseline.

PAF should measure less than 200% for acetophenone and 1-tetradecanol.

(3) Check the acidity of the column by the peak area ratios determined by integrator or triangulation of 2,6-dimethylaniline to acetophenone. A ratio of 0.7 to 1.3 for both is acceptable.

(4) Sensitivity is checked by measuring signal-to-noise ratios for the derivatives of allyl- and diallylamine. A signal-to-noise ratio of 10 to 1 for both compounds is acceptable.

Table 12.4 ACCEPTABLE SAMPLE SIZE AND CORRESPONDING DETECTION LIMITS FOR SAMPLE CONDUCTIVITY RANGES

		Detection Limit (ppb)	
Conductivity Range (ohm)	Sample Size[a] (mL)	Split Prior To Ion Exchange Concentration[b]	Split Prior to Derivatization[c]
<150	2500	1	2
150-300	1000	2	4
300-600	500	5	10
600-1,200	250	10	20
1,200-3,000	100	25	50
3,000-6,000	50	50	100
6,000-12,000	25	100	200
12,000-30,000	10	250	500

[a] Volume of sample passed through the ion exchange resin.

[b] Sample concentrate is not split prior to derivatization (split prior to ion exchange concentration).

[c] Sample concentration split immediately prior to derivatization.

Table 12.5 GC/FID OPERATING CONDITIONS FOR STRONG AMINES

GC Column	30 m DB-1 (1.0 μ film thickness) fused silica, wide bore (0.34 mm i.d.), capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through column; 15:1 split injection
Temperature Program	80 to 250°C @ 5°/min
Injector Temperature	250°C
Detector Temperature	260°C
Injection Volume	1.0 μL

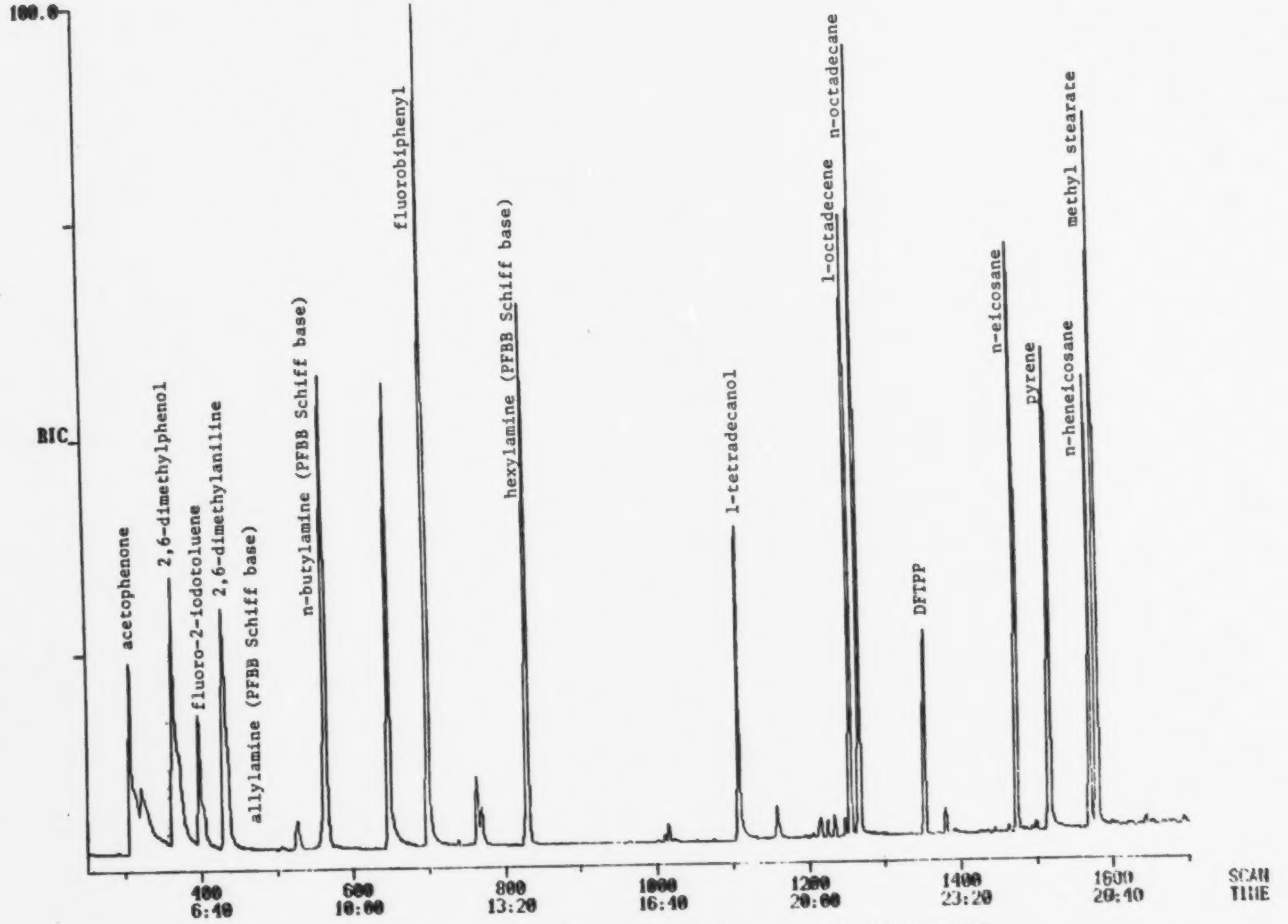


Figure 12.3. Total ion chromatogram of SAM-PT SPS.

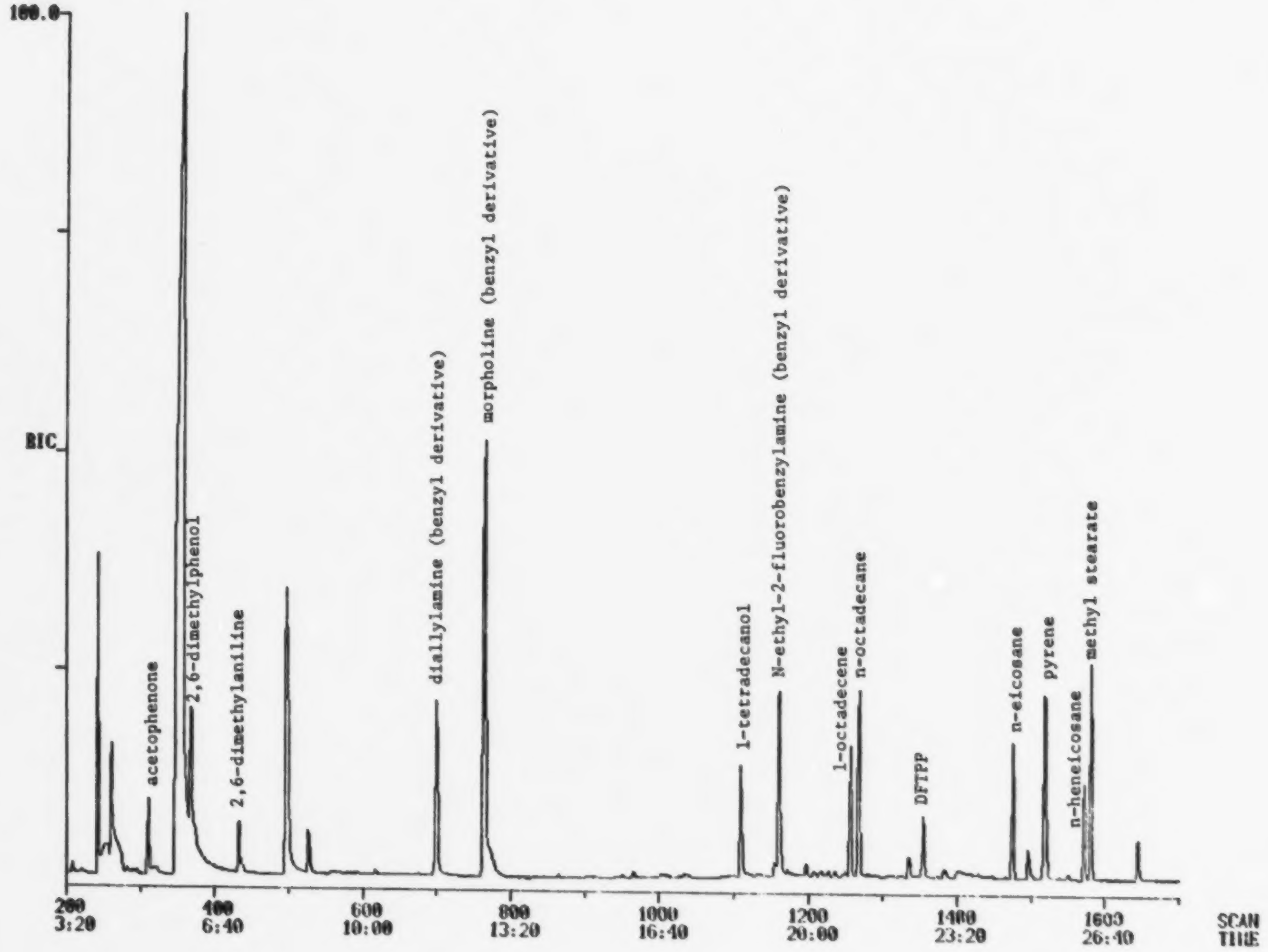


Figure 12.4. Total ion chromatogram of SAM-S SPS.

12.3.6 Derivatization and Procedural Blanks and Controls

Prior to processing any samples, a derivatization blank and a derivatization control must be run and analyzed by GC/FID to assure that background contamination is low and that derivatization yield is acceptable. After validating the derivatization step, procedural blanks must be processed and analyzed by GC/FID to detect contamination and artifacts from the resin material, solvents, reagents, dirty glassware, and other sources.

12.3.6.1 Derivatization Blanks--

(1) For the primary amine blank, add 6 mL methyl *t*-butyl ether to a 15 mL round bottom test tube. Derivatize and extract as described in step 15(a), Section 12.4.

(2) For the secondary amine standard, measure 6 mL methyl *t*-butyl ether to a 15 mL round bottom test tube. Derivatize and concentrate as described in step 15(b), Section 12.4.

(3) With a 50 µL syringe, add 10 µL of the GC external standard (hexadecane) solution to each derivatized solution.

(4) Analyze by GC/FID using the conditions described in Table 12.5. Contaminant (other than derivatizing reagents) peak heights should be less than 20% relative to the peak height of the external standard (hexadecane).

(5) If significant contamination is present, repeat the derivatization blank using fresh glassware and reagents. Reagents and solvents may be distilled or taken from another source.

12.3.5.2 Derivatization Controls--

(1) For the primary amines, prepare a control solution. With a 500 µL syringe, measure 250 µL each of the stock solutions (Table 12.3) of allylamine, *n*-butylamine, phenylethylamine, and hexylamine into a 10 mL volumetric flask and dilute to volume using pesticide grade acetone. Transfer into a Teflon sealed screw cap bottle and store at 4°C until use. For derivatization, with a 1 mL graduated pipette, measure 0.1 mL of this solution into a 15 mL round bottom test tube, add 6 mL methyl- *t*-butyl ether, and derivatize as described in step 15(a), Section 12.4.

(2) For the secondary amines, prepare a control solution. With a 500 µL syringe, measure 250 µL each of the stock solutions

(Table 12.3) of diallyamine, morpholine, and N-ethyl-2-fluorobenzylamine into a 10 mL volumetric flask and dilute to volume using pesticide grade acetone. Transfer into a Teflon sealed screw cap bottle and store at 4°C until use. For derivatization, with a 1 mL graduated pipette, measure 0.1 mL of the control solution into a 15 mL round bottom test tube, add 6 mL methyl-t-butyl ether, and derivatize as described in step 15(b), Section 12.4.

(3) With a 50 μL syringe, add 10 μL of the GC external standard to each derivative mixture.

(4) Analyze samples by GC/FID as described in Table 12.5. Peak heights of the derivatized amines measured relative to the external standard should fall within the values given in Table 12.6.

(5) If derivative yield is low (as measure by relative peak heights) this step must be repeated using fresh reagents and glassware. Reagents may be distilled or taken from another source.

12.3.6.3 Procedural Blanks--

For each batch of materials and reagents that are used, a set of three procedural blanks are required. Table 12.7 identifies these blanks and defines their purpose. These blanks must be processed and analyzed by GC/FID prior to processing any samples. If a large number of samples are to be processed, it is advantageous to prepare large batches of materials and reagents, thereby reducing the number of blanks which must be run. Procedural blank 1 must also be run every time a set of samples is processed and analyzed.

12.3.6.3.1 Procedural Blank 1--

(1) Prepare a resin column as described in step 12.4(1).

(2) Follow the procedure for amine analysis as described in step 12.4(5) to 12.4(15).

(3) With a 50 μL syringe, add 10 μL of the GC external standard to each derivative mixture.

(4) Analyze samples by GC/FID as described in Table 12.5. Contaminant (other than the derivatizing reagent) peak heights should measure less than 25% relative to the external standard.

Table 12.6. RELATIVE PEAK HEIGHTS (RPH) OF CONTROL AMINES

Amine	Acceptable RPH Range
n-butylamine	0.8-1.2
allylamine	0.8-1.2
hexylamine	0.8-1.2
diallylamine	1.5-2.0
morpholine	1.8-2.2
N-ethyl-2-fluorobenzylamine	1.5-2.0

Table 12.7. PROCEDURAL BLANKS

Blank	Description
Procedural Blank 1	Detects contamination in resin material, solvents, and glassware
Procedural Blank 2	Detects contamination in reagent water
Procedural Blank 3	Detects contamination in sulfuric acid, sodium hydroxide, and sodium thiosulfate solutions used during sample collection and analysis

(5) If significant contamination is present, the procedure should be repeated using a blank chromatography column. If this blank is acceptable, then the resin is the source of contamination and should be recleaned using the procedure in Section 12.3.2. Procedural blank 1 should then be repeated using the fresh resin material.

(6) If significant contamination is present in this blank, fresh reagents and glassware should be used and the reagent blank repeated. When the reagent blank is acceptable, procedural blank 1 must be repeated.

(7) Procedural blanks 2 and 3 may be processed once procedural blank 1 is acceptable.

12.3.6.2.2 Procedural Blanks 2 and 3--

(1) Prepare a resin column as described in step 12.4(1). Attach a separatory funnel to the top of the column. For procedural blank 2, pour 100 mL of reagent water into the separatory funnel, then drain the sample through the resin bed at a flow rate of ~10 mL/minute. For procedural blank 3, use 100 mL of reagent water spiked with 1 mL each of the sulfuric acid, sodium hydroxide and sodium thiosulfate solutions. These must be the same solutions which are to be used when the samples are collected (Chapter 5, section 5.2.14).

(2) Follow the extraction procedure as described in steps 12.4(5) to 12.4(15).

(3) With a 50 µL syringe, add 10 µL of the GC external standard to each derivative mixture.

(4) Analyze the samples by GC/FID using the conditions in Table 12.5. Peak heights for contaminants in procedural blank 3 which are not present in procedural blank 2 should measure less than 20% relative to the external standard.

(5) If significant contamination is present in procedural blank 3 which is not present in procedural blank 2, the procedure should be repeated using fresh solutions of sulfuric acid, sodium hydroxide, and sodium thiosulfate until an acceptable blank is achieved.

12.4 ION-EXCHANGE SEPARATION AND DERIVATIZATION

(1) Prepare ion-exchange resins by placing a small glass wool plug in the bottom of the chromatography column. Using a graduated pipette with the tip cut off, pipette 10 mL (allow resin to settle prior to measuring) of cleaned Biorad AG 50W-X8 resin into the open tubular chromatography column, allow the resin to settle, and rinse the column with ~10 mL of deionized water.

(2) Mix sample well (split here if necessary, see section 12.3.4). If the sample is turbid, it should stand undisturbed for 4 hours or overnight to allow particulates to settle.

(3) Gently pour the aqueous portion of the sample (equilibrated to room temperature) into a graduated cylinder to measure sample volume. Methylene chloride used as sample preservative should not be transferred. The total volume of sample collected and the volume of sample used should be recorded.

(4) For surface waters and effluents, transfer sample water to reservoir on top of the column and allow it to drain through the resin bed. Flow is gravity controlled and should be approximately 5 mL/min (NOTE: the glass wool plug can restrict flow if packed too tightly). For drinking water, transfer sample to a one gallon container. Attach siphoning adaptor between sample container and resin column. Attach a piece of tygon tubing between the end of the chromatography column and a water aspirator. To start sample flowing through the resin, open stopcock on the chromatography column and turn on aspirator. Disconnect suction after water is flowing through the resin bed. Using the stopcock, adjust flow to 2.5 mL/minute if a 2500 mL sample volume is used or 1.25 mL/minute if a 1250 mL sample volume is used. At these flow rates, it will require ~16 hours for the sample to drain through the resin bed, which is suitable for overnight accumulation. If particulate matter stops flow through the column, positive head pressure can be used to restore flow.

(5) Rinse the resin bed with 25 mL of reagent water followed by 100 mL acetonitrile. Discard these rinses.

(6) Elute the amines with 80 mL of 0.57N NaOH in 10:25 acetonitrile: water. Collect the eluate in a 200 mL round bottom flask containing 3 mL of concentrated HCl diluted with ~15 mL of reagent water.

(7) Adjust pH to <2 using additional 6N HCl if necessary. Samples can be stored refrigerated after this.

(8) Evaporate the eluate to dryness using rotary evaporation at an elevated temperature (60°C). This should take ~20 minutes. Sample must be evaporated to dryness to remove excess HCl.

(9) Resuspend the residue in 5 mL of reagent water and transfer the concentrate to a small test tube (10-15 mL). [illegible]nse the flask

with two additional 2.5 mL portions of water. Transfer rinses to the test tube. Concentrates are split at this point, if necessary (see Section 12.3.4). To split concentrates mix well. Pipette a 5 mL portion into each of two small screw cap test tubes. Similarly, split each of two rinses equally between the two test tubes. Treat one fraction as described for primary amines and one fraction as described for secondary amines. Samples can be stored refrigerated after transfer.

(10) Concentrate the solution to ~3 mL using nitrogen blowdown at an elevated temperature (60°C). Samples can be stored refrigerated at this point.

(11) Add 6 mL methyl-t-butyl ether to the test tube.

(12) Convert the amines to their free form by adding 0.2 mL of 10N NaOH. Check the pH (pH paper or meter) of the aqueous phase. Add additional NaOH until the pH of the aqueous phase is >13.

(13) Cap the tubes, and shake for 5 minutes. Vortex the tubes to remove salt particles which might cling to the sides of the tube. Centrifuge the tubes to separate the phases.

(14) Transfer the ether phase (top layer) to a second test tube for derivatization using a pasteur pipette (NOTE: Water or NaOH in the ether layer will give a significantly decreased derivative yield and cause formation of derivative by-products. Special care should be taken to avoid entraining water during this step).

(15) Derivatize samples.

(a) Primary amines

- Add 0.5 mL of the pentafluorobenzylaldehyde reagent to the methyl-t-butyl ether solution, cap the tubes with septum seal screw caps, and allow the reaction to proceed at 60°C for 90 minutes.
- Allow the reaction mixture to cool.
- Add 5 mL of 0.1N NaOH to destroy excess reagent, shake the mixture for 5 minutes, allow the layers to separate, and transfer the ether (top layer) to a small round

bottom test tube (8 mL size). Evaporate the sample to approximately 0.3 mL using nitrogen blowdown.

(b) Secondary amines

- Add 1 mL of pentafluorobenzyl bromide reagent plus 50 µL triethylamine to the methyl-t-butyl ether extract, cap the tubes with septum seal screw caps and allow the reaction to proceed at 60°C overnight. Allow the reaction mixture to cool, then evaporate the solvent to approximately 0.3 mL using nitrogen blowdown at 30°C. Since the derivatizing reagent also serves as a solvent, no further manipulations are needed. (Caution: Pentafluorobenzyl bromide is a severe lacyramator and should be used in a well ventilated hood. Although the toxicity of this reagent is unknown, exposure should be avoided).

(16) With a 50 µL syringe, add 10 µL of the MS external standard solution to each derivatized sample. Store derivatized samples at 0°C until GC/MS analysis.

12.5 GC/MS/COMP ANALYSIS

Prior to beginning GC/MS/COMP analysis of the primary and tertiary amine (SAM-PT) and secondary amine (SAM-S) fractions the analyst should be familiar with the procedures described in Chapter 13.

12.5.1 Preparation for Analysis

12.5.1.1 GC/MS/COMP Operating Parameters--

The recommended GC/MS operating parameters for the analysis of SAM-PT and SAM-S fractions are identical. They are given in Table 12.8. (The same capillary column can be used for their analysis; however, it is also recommended that the capillary used here not be used for the analysis of fractions from other protocols.)

12.5.1.2 MS Calibration--

The mass spectrometer is calibrated using the manufacturer's recommended approach (Chapter 13). The acceptability of the calibration results is verified for each SAM fraction by analyzing the appropriate system performance solution (SPS).

Table 12.8. GC/MS OPERATING CONDITIONS
FOR PRIMARY (AS THEIR SCHIFF BASES), SECONDARY (PFB DERIVATIVES),
AND TERTIARY STRONG AMINES

GC Column	30 m DB-1 (1.0 μ film thickness) fused silica wide bore (0.34 mm I.D.) capillary column
GC Carrier Gas	Helium
Carrier Gas Flow	1.6 mL/min through column; 9:1 split injection
Carrier Gas Sweep Time	85 sec (50°C)
Temperature Program	60°C to 250°C @ 4°/min and hold
Injector Temperature	250°C
Transfer Line Temperature	255°C
Injection Mode	Splitless 0.4 min/split
Injection Volume	1.0 μL
Ionizing Energy	70 eV
Ion Source Temperature	250°C
Scan Range	35-500
Scan Rate	2 sec (1.9 sec scan, 0.1 sec settling)

12.5.2 Analysis of SPS (Quality Control)

12.5.2.1 SPS for Primary/Tertiary Amines--

Using the prescribed GC/MS conditions the SPS is analyzed and all necessary data is acquired and evaluated prior to proceeding to sample analysis. The SPS is analyzed at the beginning of each day of operation or prior to starting the analysis of this fraction for the first time.

12.5.2.2 SPS for Secondary Amines--

Approach is identical as 12.5.2.1.

12.5.2.3 SPS Data Analysis--

The SPS data is extracted from the run, and test parameters calculated, and plotted or tabulated (Tables 12.9 and 12.10). Up to 14 days of SPS analytical results can be historically recorded on Tables 12.9 or 12.10 for each amine fraction set. This provides for day-to-day comparisons, and allows the analyst to follow subtle trends that may develop in

Table 12.9. GC-MS SYSTEM PERFORMANCE TEST FOR PRIMARY AND TERTIARY AMINES (SAM-PT)

Dates: ________ ________ Run Id Code ________ ________
________ ________ ________ ________
________ ________ ________ ________
________ ________ ________ ________
________ ________ ________ ________
________ ________ ________ ________
________ ________ ________ ________

GC Column and Program: ____
____

Notebook References: ____
____
____

MS SYSTEM CHECKS

High/Low Mass Balance Ion	DFTPP Relative Abundance Criteria
51	30% to 60% of m/z 198
68	<2% of m/z 69
70	<2% of m/z 69
127	40% to 60% of m/z 198
197	<1% of m/z 198
198	100%
199	5-9% of m/z 198
275	10% to 30% of m/z 198
365	at least 1% of m/z 198
441	present, but < m/z 443
442	>40% of m/z 198
443	17% to 23% of m/z 442

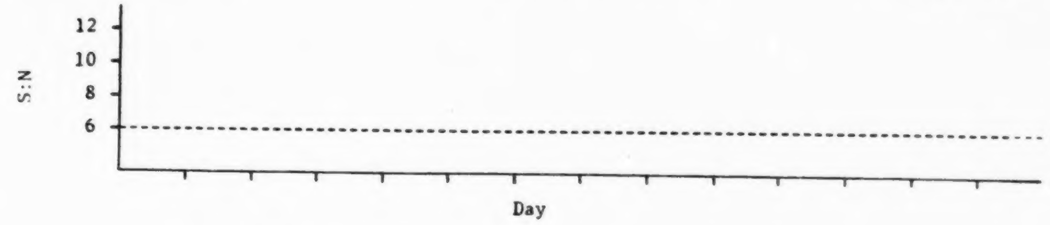
m/z	51	68	70	127	197	198	199	275	365	331	442	443	Pass/ Fail	Remedial Action
Abundance * Criteria	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )		
Day														

*These abundances are initially established by the MAS user, and subsequently become guidelines for acceptability of tune.

(continued)

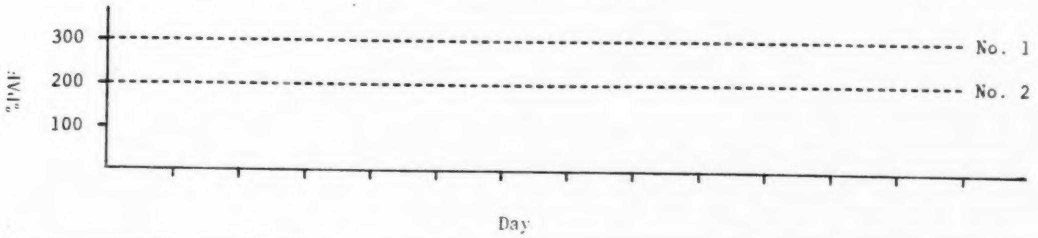
Table 12.9 (cont'd.)

Test	Components	Criteria
Limit of Detection	Allylamine (as Schiff's Base)	S:N (m/z 207) > 6:1

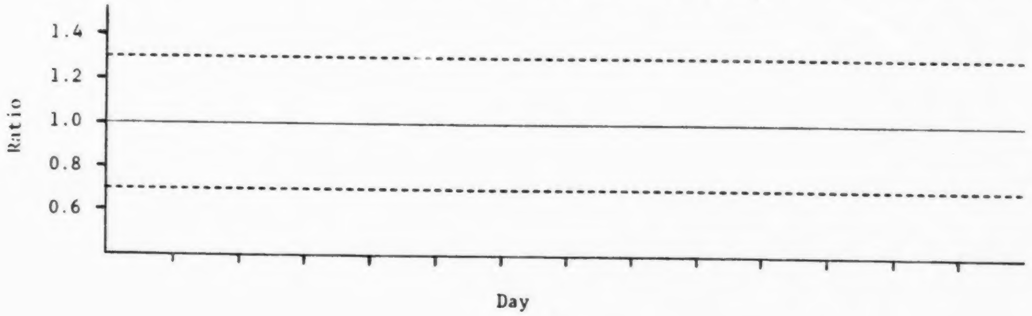


GC SYSTEM CHECKS

Test	Components	Criteria
Peak Asymmetry	1. Acetophenone (TIC) ● 2. 1-Tetradecanol (TIC) ■	%PAF <300 %PAF <200

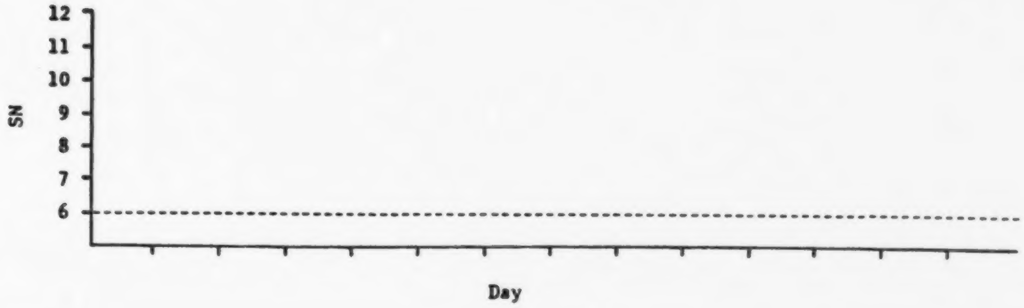
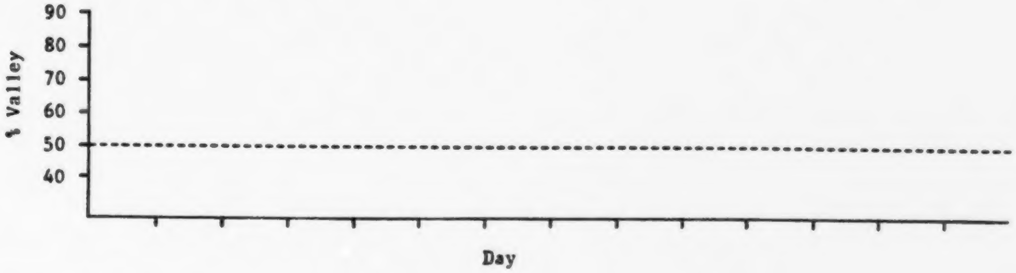
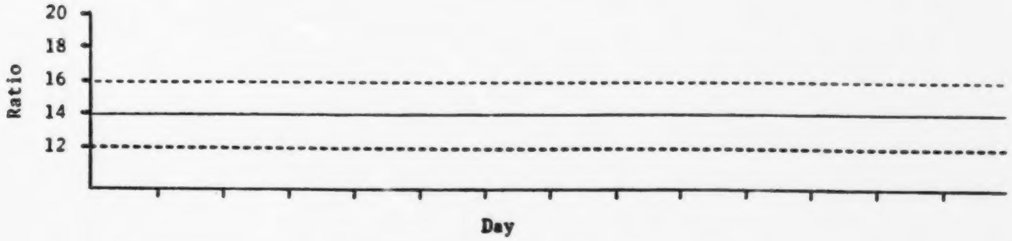


Test	Components	Criteria
Acidity	1. Acetophenone (TIC) 2. 2,6-Dimethylaniline (TIC)	Ratio 2:1 = 0.7 to 1.3



(continued)

Table 12.9 (cont'd.)

Test	Components	Criteria
Separation Number	n-Eicosane (m/z 43) n-Heneicosane (m/z 43)	SN ≥ 6
		
Resolution	n-Octadecane (TIC) 1-Octadecene (TIC)	R = minimum 50% Valley
		
Inertness	Methyl Stearate	Ratio m/x 74 to 298 = 12:1 to 16:1
		

(continued)

Table 12.9 (cont'd.)

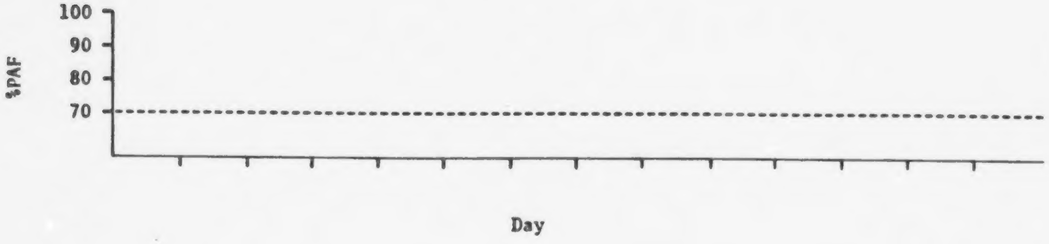
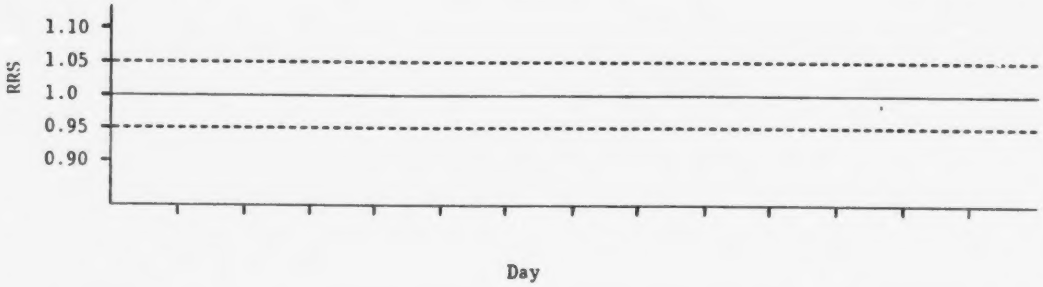
Test	Components	Criteria
Capillary Capacity	Hexylamine (as Schiff's Base) (TIC)	%PAF >70
		
Relative Retention Shift	1. Heneicosane 2. Pyrene	RRS = 0.95-1.05
		

Table 12.10. GC-MS SYSTEM PERFORMANCE TEST FOR SECONDARY AMINES (SAM-S)

Dates: ______ ______ **Run Id Code** ______ ______

GC Column and Program: ____________________

Notebook References: ____________________

MS SYSTEM CHECKS

High/Low Mass Balance Ion	DFTPP Relative Abundance Criteria
51	30% to 60% of m/z 198
68	<2% of m/z 69
70	<2% of m/z 69
127	40% to 60% of m/z 198
197	<1% of m/z 198
198	100%
199	5-9% of m/z 198
275	10% to 30% of m/z 198
365	at least 1% of m/z 198
441	present, but < m/z 443
442	>40% of m/z 198
443	17% to 23% of m/z 442

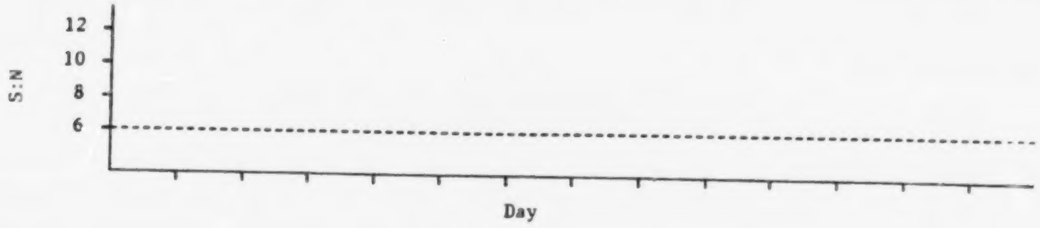
m/z	51	68	70	127	197	198	199	275	365	331	442	443	Pass/ Fail	Remedial Action
Abundance * Criteria	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )	( )		
Day														

*These abundances are initially established by the MAS user, and subsequently become guidelines for acceptability of tune.

(continued)

Table 12.10 (cont'd.)

Test	Components	Criteria
Limit of Detection	Diallylamine (as PFB Derivative)	S:N (m/z 250) > 6:1

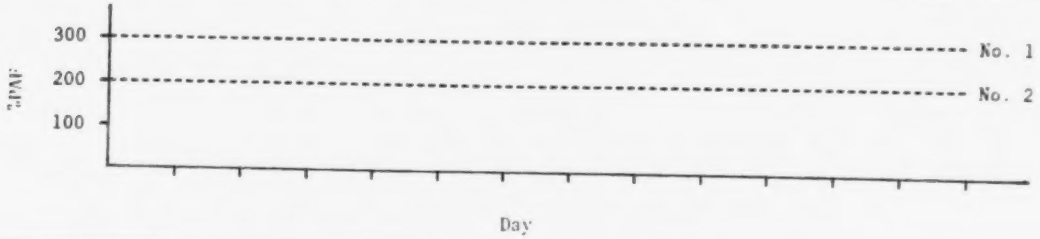


GC SYSTEM CHECKS

Peak Asymmetry

1. Acetophenone (TIC) ●
2. 1-Tetradecanol (TIC) ■

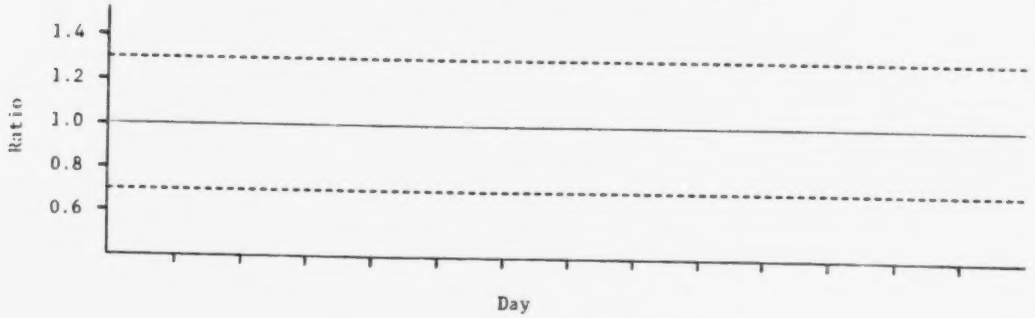
%PAF <300
%PAF <200



Acidity

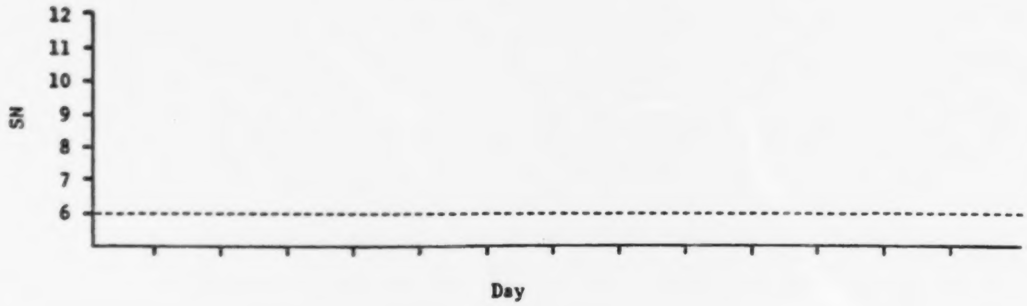
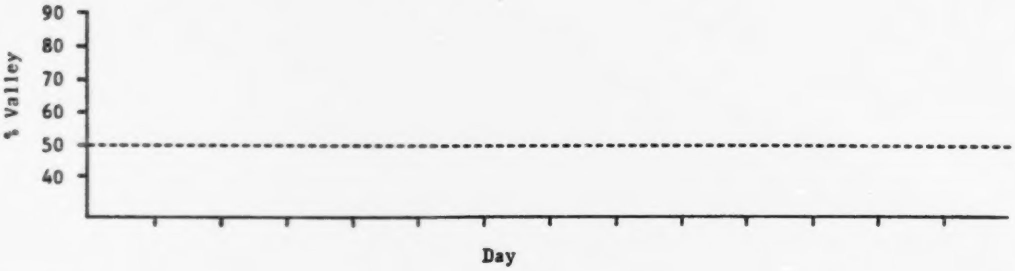
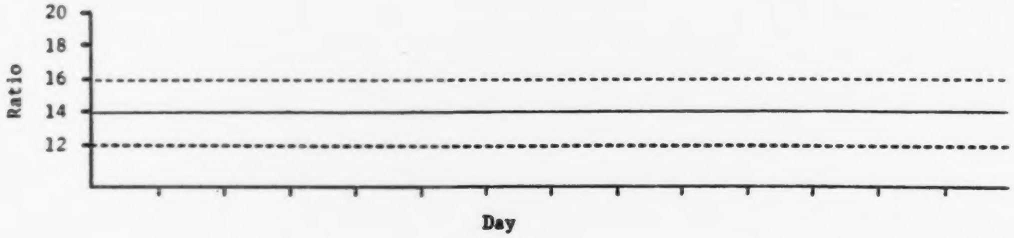
1. Acetophenone (TIC)
2. 2,6-Dimethylaniline (TIC)

Ratio 2:1 = 0.7 to 1.3



(continued)

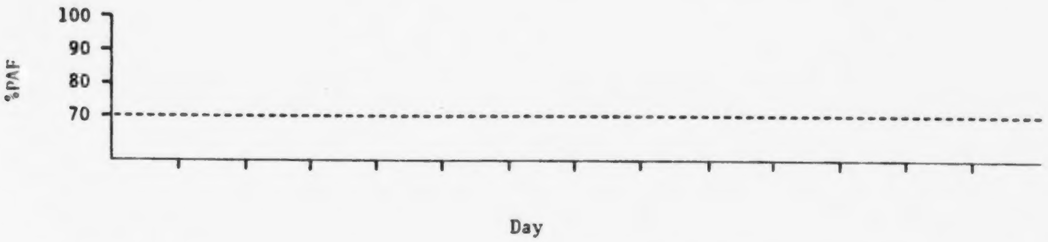
Table 12.10 (cont'd.)

Test	Components	Criteria
Separation Number	**n-Eicosane (m/z 43)** **n-Heneicosane (m/z 43)**	**SN ≥ 6**
		
Resolution	n-Octadecane (TIC) 1-Octadecene (TIC)	**R = minimum 50% Valley**
		
Inertness	**Methyl Stearate**	Ratio m/x 74 to 298 = 12:1 to 16:1
		

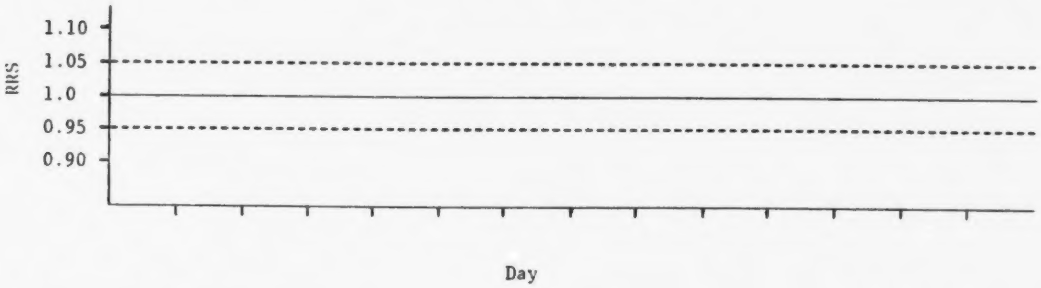
(continued)

Table 12.10 (cont'd.)

Test	Components	Criteria
Capillary Capacity	Morpholine (as PFB Derivative) (TIC)	%PAF >70



Test	Components	Criteria
Relative Retention Shift	1. Heneicosane 2. Pyrene	RRS = 0.95-1.05



anticipation of GC or MS maintenance requirements. Chapter 13 explains calculations and usage of these data.

Daily RMR checks are also made and recorded in Table 12.11 or 12.12, depending on the amine fraction undergoing analysis. These RMRs are compared to those obtained during the development of the historical data bank. The usage of these data is also discussed in Chapter 13.

12.5.3 Determination of RMRs

RMRs for the User's data bank (as opposed to the daily RMR checks discussed in the above paragraph) are determined by injecting a solution containing the derivatized internal standards and analytes.

Table 12.11. GC-MS SYSTEM PERFORMANCE TEST: RMR CHECK FOR PRIMARY AND TERTIARY AMINES

Date: ____________________ Run ID Code: ____________________

DATA

Standard	MW	Amount (ng)	µM	Ion (m/z)	Area (Run 1)	Area (Run 2)
4-Fluoro-2-iodo-toluene (FIT)	236			109		
				236		
2-Fluorobiphenyl	172			172		
d_9-butylamine	261			210		
				212		
d_4-phenylethylamine	304			304		

MATRIX OF STANDARD ION RMRs

Standard	Ion	109	236	172	210	212	304
FIT	109	-					
	236		-				
2-Fluorobiphenyl	172			-			
d_9-butylamine	210				-		
	212					-	
d_4-phenylethylamine	304						-

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

Table 12.12 GC-MS SYSTEM PERFORMANCE TEST:
RMR CHECK FOR SECONDARY AMINES (SAM-S)

Date: ____________________ Run ID Code: ____________________

DATA

Standard	MW	Amount (ng)	µM	Ion (m/z)	Area (Run 1)	Area (Run 2)
2-Fluorobiphenyl	172			172		
4-Fluoro-2-iodo-toluene (FIT)	236			109		
				236		
N-Ethyl-2-fluoro-benzylamine	314			314		

MATRIX OF STANDARD ION RMRs

Standard	Ion	172	109	236	314
2-Fluorobiphenyl	172	-			
FIT	109		-		
	236			-	
N-Ethyl-2-fluoro-benzylamine	314				-

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

The GC/MS/COMP operating parameters are given in Table 12.8. Table 13.8 may be used to tabulate RMR raw data.

12.5.4 Analysis of Field and Quality Control Samples

After all specified criteria for GC and MS performance (Table 12.9 or 12.10 and 12.11 or 12.12) have been met, analysis of standard solutions, QA/QC samples, (e.g., for RMR calculations) or SAM-PT/SAM-S sample extracts is conducted.

Qualitative and quantitative procedures are described in Chapter 13. Tables 12.13 and 12.14 may be used to tabulate sample analyte raw data for the SAM-PT and SAM-S fractions, respectively, prior to using the MASQUANT software program or manual quantitative calculations.

Table 12.13. RAW ANALYTE DATA FOR SAM-PT FRACTION

Date: __________ Run I.D. Code: __________

Sample Identification: __________ Notebook Reference: __________

Vol. Water (L): __________ Vol. Water Processed (mL): __________
(to which iS were added)

STANDARDS

	I.D. No.	Spectrum No.	Weight (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
4-Fluoro-2-iodotoluene				109,236:	,		,
2-Fluorobiphenyl				172:	,		,
d_9-Butylamine				210,212:	,		,
d_4-Phenylethylamine				304:	,		,

ANALYTES Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Area	MW_x^a
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	
			, :	,	

---

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{RMR_{x/y}} \times \frac{ng_y}{A_y \cdot MW_y}$$

$$K = \frac{ng_y}{A_y \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

Table 12.14. RAW ANALYTE DATA FOR SAM-S FRACTION

Date: ____________

Run I.D. Code: ____________

Sample Identification: ________

Notebook Reference: ____________

Vol. Water (L): ____________
(to which iS were added)

Vol. Water Processed (mL): ____

STANDARDS

	I.D. No.	Spectrum No.	Weight (ng_y)	Ions (m/z)	Area	MW_y^a	K^a
2-Fluorobiphenyl	___	___	___	172:	___, ___	___	___, ___
4-Fluoro-2-iodotoluene	___	___	___	109,236:	___, ___	___	___, ___
N-Ethyl-2-fluorobenzylamine	___	___	___	314:	___, ___	___	___, ___

ANALYTES

Class No. ______

	I.D. No.	Spectrum No.	Ions (m/z)	Area	MW_x^a
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___
____________	___	______	___, ___:	___, ___	___

[a] If calculations are performed manually then this information is also needed, whereas MASQUANT performs functions automatically.

$$ng_x = \frac{A_x \cdot MW_x}{RMR_{x/y}} \times \frac{ng_y}{A_y \cdot MW_y}$$

$$K = \frac{ng_y}{A_y \cdot MW_y}$$

A = ion area or height
ng = ng recovered from volume of water processed
x = analyte
y = standard

CHAPTER 13

GC/MS/COMP ANALYSIS PROCEDURES - GENERAL INSTRUCTIONS FOR ALL PROTOCOLS

13.1 INTRODUCTION

This chapter discusses the general approach and procedures for qualitative and quantitative analysis of sample fractions derived from each MAS analytical protocol. Also, an example of calculations of the concentrations of analytes in a water sample are given. Once the analyst has become acquainted with this chapter and skillful with the instrumental analysis steps, he must consult the individual analytical protocol to obtain further detailed information specific to the fraction (extract) to be analyzed.

The application of a good quality assurance/quality control (QA/QC) program is essential to ensure the validity of data obtained from the GC/MS/COMP system. As part of the QA/QC program, the Master Analytical Scheme incorporates the use of system performance standards (SPS) to assure the proper operation of the GC/MS/COMP system. The instrument characteristics, especially those of the capillary column, are tested with the SPS using established procedures (Grob, K., et al., J. Chromatogr., 156, 1, 1978). The criteria given for column performance in each analytical protocol are based on "safe" limits. As the Scheme is used for a variety of water types and sample fractions, the information gathered will permit the user to adjust the boundaries of the limits for each criterion according to his own application needs.

13.2 APPARATUS AND MATERIALS

This section describes the general apparatus and materials which will be required to perform instrumental analysis of fractions produced by each of the protocols in the Master Analytical Scheme. These are:

(1) Combined gas chromatograph/mass spectrometer with the following capabilities:

- (a) scan range of 35-600 daltons (maximum, range needed varies with specific protocol);
- (b) scan cycle of 2 sec or faster (start-to-start);
- (c) at least unit resolution through the mass range;
- (e) GC injector (Grob or Grob-type) capable of split and split/ splitless injection modes;
- (f) capability of direct coupling of capillary column to the ion source or of coupling through a separator with make-up gas; and
- (g) computer with, as a minimum, a software package capable of acquiring continuous mass scans, extracting selected ion current, quantifying ions by peak area (preferable) or peak height (acceptable) and equipped with an ANSI Fortran compiler.

(2) Gas chromatographic column - fused silica or glass capillary column, wide bore, with thick film. The exact length, internal diameter, stationary phase coating and thickness are prescribed in each analytical protocol.

(3) System Performance Solution - contains a series of compounds to assess the tuning and chromatographic performance of the GC/MS system. Each SPS is tailored to the specific fraction generated by an analytical protocol. An example is given in Table 13.1 for the ESSA fraction.

(4) Solutions for generating RMRs for all MAS fractions, containing target compounds of interest:

- (a) ~400 ng/μL in methylene chloride, for all compounds except very volatile VO and NEWS;
- (b) ~400 ng/μL in water, for elevated temperature purge and trap (NEWS) compounds;
- (c) in gas-phase mixture, for very volatile VO organics (those which cannot be put into methylene chloride solution).

(5) Ultra-pure helium, associated clean lines and two-stage stainless steel diaphragm pressure regulator.

(6) 10 μL syringe.

Table 13.1. GC/MS SYSTEM PERFORMANCE SOLUTION FOR ESSA

Compound	Density (@ 20°C)	Concentration µg/mL in Methanol
2,6-dimethylphenol	S[a]	300
acetophenone	1.030	310
1-tetradecanol	0.823	250
1-octadecene	0.789	230
_n_-octadecane	0.777	231
DFTPP	S	500
_n_-eicosane	S	300
pyrene	S	300
_n_-heneicosane	S	300
methyl stearate	S	10
d_{13}-heptanoic acid (methyl ester)	0.948	280
d_5-benzoic acid (methyl ester)	1.13	340
4-fluoro-2-iodotoluene	0.883	260
methyl decanoate	0.873	870

[a] Solid.

13.3 OPERATIONAL PARAMETERS

13.3.1 Mass Spectrometer Operating Conditions and Tuning

For a quadrupole mass spectrometer, a 0.1 sec hold at low mass and a scan cycle of ∿1 sec (0.9 sec scan, 0.1 sec settling time at low mass) for the Finnigan 4021 (2 msec/mass integration time) and 2 sec (1.9 sec scan, 0.1 sec settling time at low mass) for the Finnigan 3300 (4 msec/mass integration) are used. All spectra are recorded at 70 eV. The transfer line (if capillary is not directly coupled to the ion source) is maintained at 280°C (or at a temperature to allow satisfactory transmission of pyrene).

The complete mass spectra are recorded from mass 40 to mass 450. All data recorded are archived on IBM-compatible 9-track magnetic tapes for future reference.

The system is given an initial tune each day of operation (or as needed) to obtain a good signal level, acceptable peak shape, at least unit resolution through the mass range, and calibration of the mass range according to the MS manufacturer's recommended procedure. The system can be given this preliminary tune by the use of perfluorotributylamine, perfluorokerosene, tris(perfluoroheptyl)-s-triazine or any other standard which is in routine use in a given laboratory. The standard should be introduced through a molecular leak or some other type of reservoir system which will maintain a stable low steady-state concentration of the material in the ion source (similar to pressures achieved under GC conditions) during the time adjustments to the system are being made. The mass spectrometer inlet system used for the introduction of the tuning compound should allow an easily reproducible quantity of the standard compound to be introduced in order to avoid any possible discrimination effects which could arise from differences in source pressures on different days.

13.3.1.1 Purgeable Protocols (VO and NEWS fractions)--

The ability of the tuned system to meet specific MAS criteria for analysis of volatile organics can be evaluated by the introduction of perfluorotoluene (PFT) and 1-bromo-4-fluorobenzene (BFB) through the same inlet system used for perfluorokerosene (or other tuning standard). This tune, however, is ultimately confirmed by the spectrum of PFT and BFB, which are components of the system performance solution, obtained when the system performance solution is chromatographed. Mean relative abundances of major ions of the PFT and BFB spectra obtained under one set of conditions are shown in Table 13.2 and 13.3, respectively. The user should be able to tune his instrument to a stable point within the percent relative standard deviation and thereafter maintain his tune to within ±15%. The exact position of stable tune on any user's instrument must be determined experimentally. The resulting PFT or BFB ion distribution should be attainable under normal operating conditions and the instrument must be stable (±15%) for at least an eight-hour day. (If a double or triple shift day is used, tune stability

Table 13.2. RELATIVE INTENSITIES OF IONS IN PERFLUOROTOLUENE[a]

m/z	Relative Intensities (CV)
69	33 (5)
79	11 (10)
93	16 (8)
117	43 (8)
167	15 (7)
186	59 (5)
217	100
236	66 (4)
237	5

[a] Data obtained on Finnegan 4021 GC/MS/DS; 60 m wide-bore (0.35 mm) thick film (1 μ) fused silica DB-1 column; isothermal hold, 5 min at 40°C, then programmed to 200°C at 4°C/min.

Table 13.3. RELATIVE ABUNDANCES OF BFB IONS

Ion	Relative Abundance
50	15-40% of mass 95
75	30-60% of mass 95
95	100%
96	5-9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5-9% of mass 174
176	>95%, <101% of mass 174
177	5-9% of mass 176

should be checked every eight hours, until sufficient data are accumulated to show that such frequent checking is unnecessary.)

13.3.1.2 Other Protocols--

The ultimate check on the tune of the system for all fractions except VO and NEWS is made using decafluorotriphenylphosphine (DFTPP) as indicated in the Federal Register, 44, 69536 (12/3/79). DFTPP must be chromatographed in order to assess correspondence of the observed relative abundances of ions with those reported in the Federal Register, and presented in Table 13.4. Many methods can be used to obtain the mass spectrum from a chromatographic peak: a spectrum may be taken at the top of the peak, a background (before or after the peak) may be subtracted, an averaged spectrum (by several different techniques) may be used, or an averaged spectrum with subtracted background may be obtained. The exact technique used in obtaining the spectrum is less important than ensuring that the same method is used consistently for a given instrument. Each MAS system performance solution

Table 13.4. RELATIVE ABUNDANCES OF DFTPP IONS[a]

Ion	Relative Abundance
51	30-60% of mass 198
68	less than 2% of mass 69
70	less than 2% of mass 69
127	40-60% of mass 198
197	less than 1% of mass 198
198	base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	at least 1% of mass 198
441	present, but less than mass 443
442	greater than 40% of mass 198
443	17-23% of mass 442

[a] Federal Register, 44, 69536 (12/3/79).

(except those for VO and NEWS fractions) contains DFTPP and is used to obtain and check the DFTPP spectrum. The relative abundances of the ions of DFTPP are to conform to the ranges presented in Table 13.4. (An example is given in Table 13.5.) Precision for the user's ion abundances should be ±15% within one eight-hour day's operation.

13.3.2 GC Operating Conditions

The specific capillary column to be used with each protocol is given under Section 13.5 of each protocol. The GC operating conditions are also specifically stated for analysis of extracts/fractions produced by a protocol.

If glass capillaries that meet performance specifications are employed rather than fused silica, then the following guidelines are given for straightening their ends for installation:

(1) a low flow (1-2 mL/min) of an inert gas is passed through the column toward the end to be straightened;

Table 13.5. MEAN RELATIVE ABUNDANCES OF MAJOR IONS OF DFTPP OBSERVED IN A SYSTEM PERFORMANCE SOLUTION [a]

Ion	Relative Abundance	C.V.
51	31	3
68	0.3	8
69	31	3
70	0.6	7
77	40	2
110	23	1
127	41	2
197	0.3	12
198	100	-
199	8	7
206	17	1
255	30	1
275	19	2
365	2	15
441	3	29

(continued)

Table 13.5 (cont'd.)

Ion	Relative Abundance	C.V.
442	45	4
443	7	4

[a] Data obtained on Finnegan 4021 GC/MS/DS; column, 30 m DB-1 wide-bore (0.32 mm) thick film (1 μ) fused silica capillary; 50°C for 5 min, then programmed to 240°C at 4°C/min.

(2) the column is held so that the portion to be straightened will point down when straightened;

(3) a yellow flame (from, e.g., the base of a Bunsen burner), just hot enough to allow the glass to straighten but not melt, is passed along the column from the point where the straight portion is to start to the end of the column, allowing the weight of the glass to straighten the end as it is heated;

(4) a 0.1% Carbowax 20M solution in CH_2Cl_2 is injected into the straightened end of the capillary (using a 50 μL syringe connected to the column end with Teflon tubing) against the inert gas flow until it fills the portion of the column that has been heated in the flame;

(5) the 0.1% Carbowax 20M solution is withdrawn and the syringe disconnected;

(6) the inert gas flow is maintained until any remaining solvent is evaporated;

(7) the inert gas flow is stopped; and

(8) the last 2-3 cm of the column is broken off.

After installing the capillary column, the carrier flow is optimized according to the capillary column manufacturer's instructions. A typical flow rate would be 2.0 mL/min (~18 cm/sec) for a 0.32 mm i.d. fused silica capillary. See each MAS protocol for specific flow rates.

The GC injection system should be capable of split and split/splitless modes. The split/splitless mode must be optimized for each fraction as per manufacturer's instructions. Splitter and carrier gas flows must, of course, be adjusted when the GC/MS system is set up for a given MAS protocol. At the initial stages of operation in a given protocol, the accuracy of the gas flows should be verified for every 24 h period. After the user has established the stability of flows, it may be sufficient to check less frequently (perhaps weekly).

An injection size of up to 2.0 μL is used for a split injection and, for splitless, up to 1.0 μL. The injection port is maintained at 250°C.

Approximately once a month, during the analysis of a SPS, the GC oven temperature readout should be verified using an alternate measuring device such as a thermometer or digital thermometer. The rate of rise of the oven temperature at the various temperature program rates should also be verified.

13.4 ANALYSIS OF SYSTEM PERFORMANCE SOLUTION (SPS) AND SAMPLES

After establishing mass spectrometer tuning and GC conditions, the first GC/MS analysis performed each day is on the appropriate SPS. The data acquired from the SPS analysis are examined to determine whether the MS and GC performance is acceptable for sample analysis. Then, extracts/fractions are analyzed. The extracts/fractions are grouped as sets (e.g., all extractable acid, ESSA extracts from one series of water samples is a set) and analyzed by GC/MS.

This section provides general comments and procedures, which are prescribed more specifically in each of the analytical protocols, for the analysis of SPS and sample extracts. Specific case examples are given.

13.4.1 SPS - Data Acquired and Calculations

The following information is needed from the SPS analysis for subsequent calculations:

(1) Perfluorotoluene (PFT), 1-bromo-4-fluorobenzene or decafluorotriphenylphosphine (DFTPP) spectrum, as specified by the analytical protocol;

(2) Reconstructed total or extracted ion current profiles for the other SPS components;

(3) Retention time (spectrum no.) for each SPS component; (Spectrum number is used because this is the parameter used by MASQUANT to determine which of the internal standards elutes closest to the target compound of interest. No generation of relative retention

times (RRT) as a separate data base is being done. If the user desires an RRT data base, he may use ratios of spectrum numbers or absolute retention times at peak tops.);

(4) Areas for selected ions of certain components (usually the internal and external standards) for calculation of RMRs to correct for tuning differences (see Section 13.4.2.2.3) if MS tune cannot meet criteria.

13.4.1.1 Acceptability of MS Tune--

The abundance of each appropriate ion for PFT, BFB, or DFTPP (depending upon fraction to be analyzed) that is obtained from analyzing the SPS is tabulated for inspection and a historical record (for example, Table 13.6). The analyst is to initially establish the precise abundance for these ions on his instrument by analyzing PFT, BFB, or DFTPP a minimum of six times, preferably over several days of operation. This mean average abundance for each ion is recorded as, for example, on the chart at the bottom of Table 13.6 in the "blank" parentheses (line labeled "Abundance"). Subsequently, the analysis of the SPS (prior to analysis of MAS fractions) is conducted daily the PFT or DFTPP ion abundances listed for each day's operation. Acceptability of tune is demonstrated by the maintenance of ion abundance within ±15% relative standard deviation of the analyst's established guidelines.

An example of data obtained for the SPS of extractable acids (ESSA) is given in Table 13.6.

Failure to meet criteria requires one or all of the following: (a) retuning; (b) cleaning the source (and rods, if necessary); or (c) other maintenance to reestablish acquisition of data within performance specifications. All reasonable efforts should be made to bring the instrument within performance specifications. If, for some reason (time constraints, sample storage deadlines), the system cannot be made to meet specifications, see Section 13.4.2.2.3 for a method for correcting relative molar response values.

After criteria of ion abundance for PFT or DFTPP have been met the chromatographic performance characteristics for the SPS analysis are examined.

Table 13.6. GC-MS SYSTEM PERFORMANCE TEST FOR EXTRACTABLE SEMIVOLATILE STRONG ACIDS (ESSA)

Dates:			Run Id Code		
8-7-81	8-17-81			ESSA-1	ESS-12
8-8-81				ESSA-2	
8-9-81				ESSA-3	
8-10-81				ESSA-4	
8-11-81				ESSA-5	
8-15-81				ESSA-8	
8-16-81				ESS-10	

GC Column and Program: 30m DB-1 wide bore, 1.0μ film: 50-240°, 3°/min

Notebook References: 1691-3,4

MS SYSTEM CHECKS (Injection of 1 μL of SPS)

High/Low Mass Balance Ion	DFTPP Relative Abundance Criteria
51	30% to 60% of m/z 198
68	<2% of m/z 69
70	<2% of m/z 69
127	40% to 60% of m/z 198
197	<1% of m/z 198
198	100%
199	5-9% of m/z 198
275	10% to 30% of m/z 198
365	at least 1% of m/z 198
441	present, but < m/z 443
442	>40% of m/z 198
443	17% to 23% of m/z 442

m/z	51	68	70	127	197	198	199	275	365	331	442	443	Pass/ Fail	Remedial Action
Abundance	(31)*	(1.5)	(1.8)	(41)	(.7)	(100)	(7)	(25)	(1.1)	(7)	(44)	(9)		
Day 8-7	31	1.4	1.8	41	.7	100	7	25	1.1	7	44	8	P	—
8-7	35	1.4	1.9	47	.9	100	9	28	1.4	7	47	9	P	—
8-8	29	1.6	1.7	39	.6	100	6	28	1.3	7	35	8	F	Retune
8-8	31	1.5	1.8	41	.7	100	7	25	1.1	8	44	9	P	—
8-9	29	1.7	1.7	39	.6	100	6	27	1.2	7	34	8	F	Retune
8-9	30	1.5	1.7	40	.7	100	7	24	1.1	7	43	8	P	—
8-10	30	1.5	1.7	40	.7	100	7	24	1.1	7	43	9	P	—
8-11	31	1.5	1.8	41	.7	100	7	25	1.1	7	44	8	P	—
8-15	32	1.6	1.8	42	.8	100	8	26	1.2	7	45	9	P	—
8-16	30	1.6	1.7	40	.7	100	7	24	1.1	7	43	8	P	—
8-17	31	1.5	1.8	41	.7	100	7	25	1.1	8	44	9	P	—

*These abundances are initially established by the MAS user, and subsequently become guidelines for acceptability of tune.

(continued)

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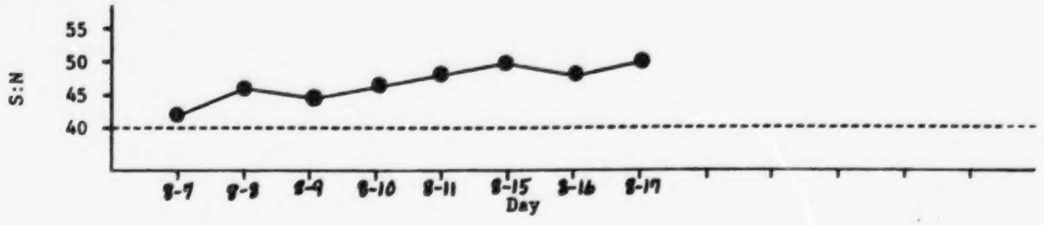
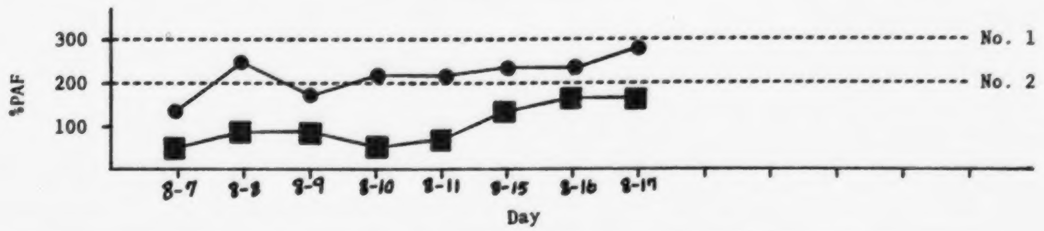
Table 13.6 (cont'd.)

Test	Components	Criteria
Limit of Detection	Methyl Stearate	S:N (m/z 74) > 40:1

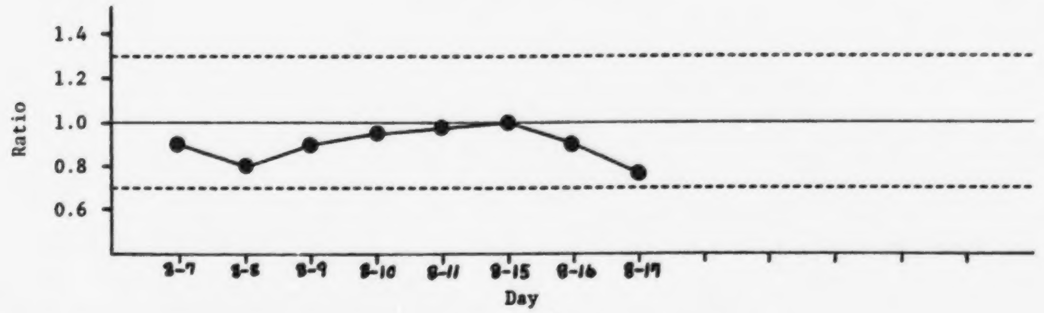


GC SYSTEM CHECKS

Test	Components	Criteria
Peak Asymmetry	1. Acetophenone (TIC) 2. 1-Tetradecanol (TIC	%PAF <300 %PAF <200



Test	Components	Criteria
Basicity*	1. Acetophenone (TIC) 2. 2,6-Dimethylphenol (TIC)	Ratio 2:1 = 0.7 to 1.3



*Note: Acidity criteria are also measured in other MAS fractions.

(continued)

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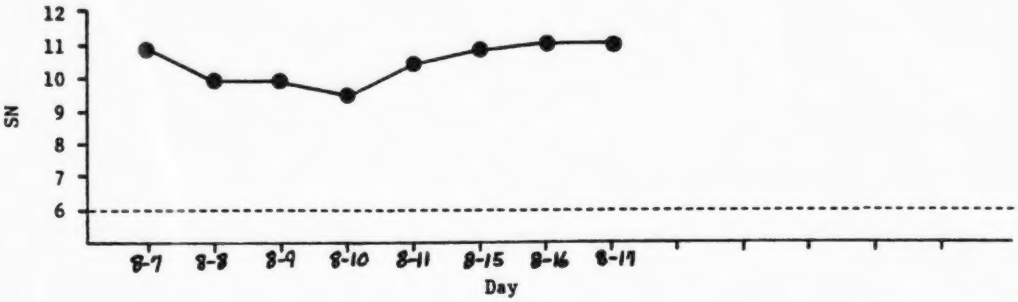
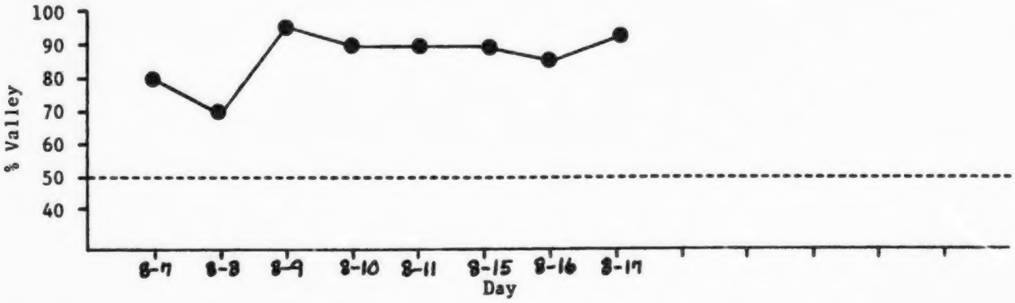
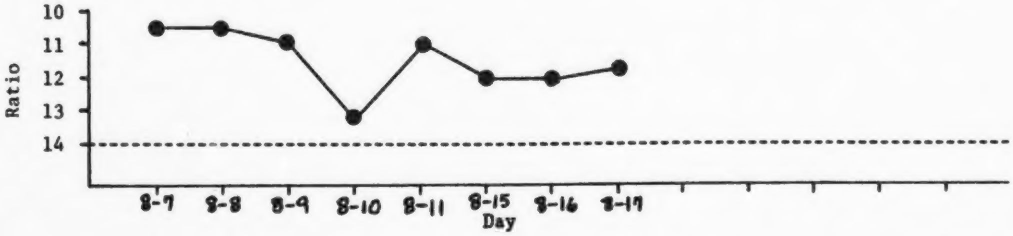
Table 13.6 (cont'd.)

Test	Components	Criteria
Separation Number	n-Eicosane (m/z 43) n-Heneicosane (m/z 43)	SN ≥ 6
Resolution	n-Octadecane (TIC) 1-Octadecene (TIC)	R = minimum 50% Valley
Inertness	Methyl Stearate	Ratio m/z 74 to 298 ≤ 14:1



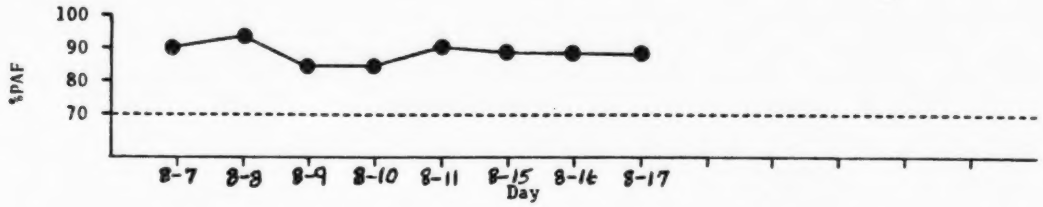




(continued)

Table 13.6 (cont'd.)

Test	**Components**	**Criteria**
Capillary Capacity	**Methyl Decanoate (TIC)**	**%PAF >70**



13.4.1.2 Peak Asymmetry--

The peak asymmetry or tailing is measured by the percent peak asymmetry factor (PAF):

$$\% \text{ PAF} = \frac{B}{F} \times 100$$

where B = the width of the back half of a chromatographic peak measured at 10% above baseline

F = width of the front half of the chromatographic peak measured at 10% above baseline

An example of this measurement is given in Figure 13.1 for acetophenone. Specific criteria are given with each analytical protocol, e.g., see Table 13.6.

13.4.1.3 Acidity/Basicity--

The acidity/basicity of the column is checked by the peak area ratios (determined by computer or triangulation) of 2,6-dimethylaniline and/or 2,6-dimethylphenol to acetophenone. A ratio of 0.7 to 1.3 for both is acceptable. A typical measurement is given in Figure 13.2. Specific criteria are given with each analytical protocol, e.g., see Table 13.6.

13.4.1.4 Separation Number--

The separation number (SN) is a measure of the separation efficiency of the column and is determined as follows:

$$SN = \frac{D}{W_1 + W_2} - 1$$

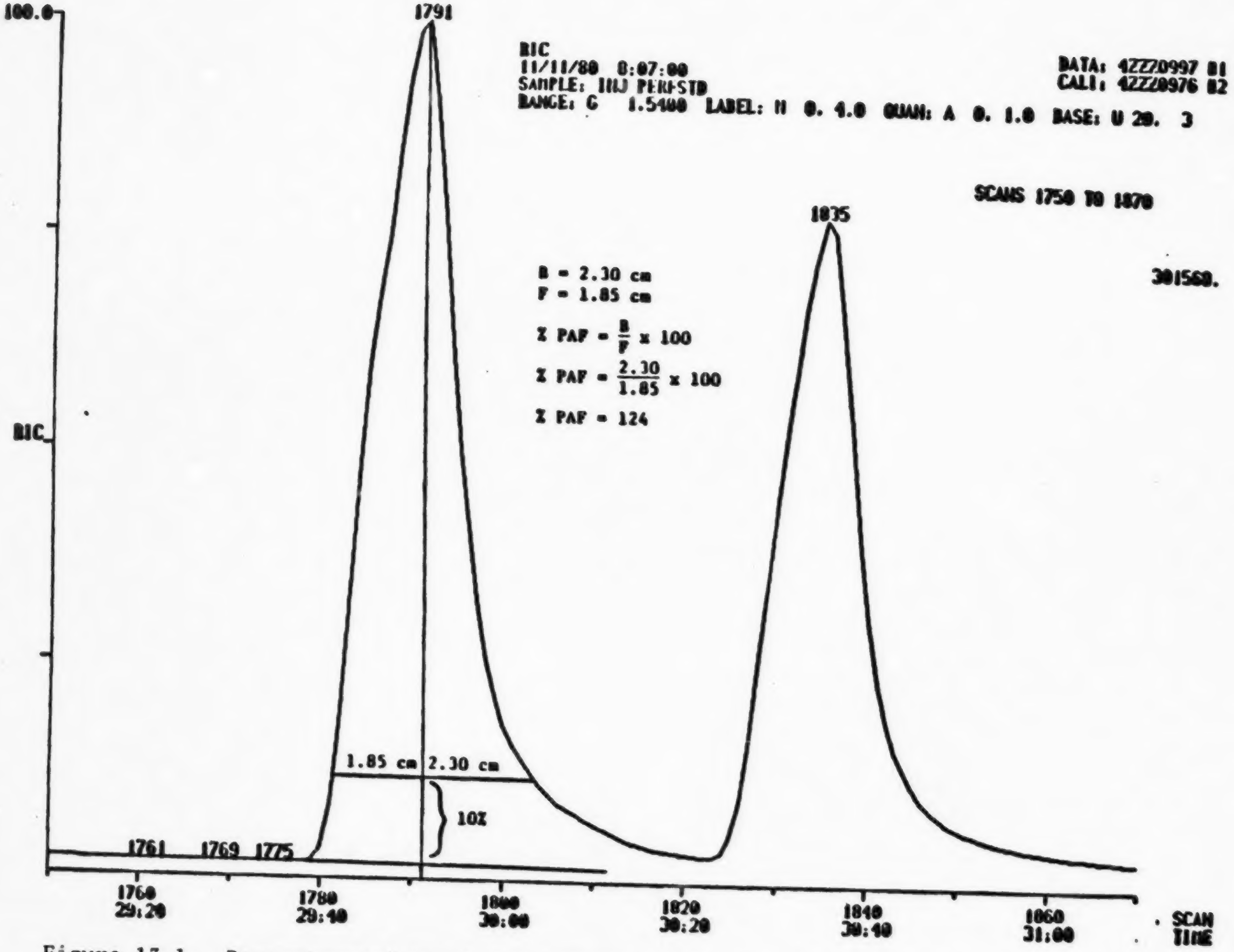


Figure 13.1. Reconstructed total ion current profile of acetophenone used in calculating percent peak asymmetry factor.

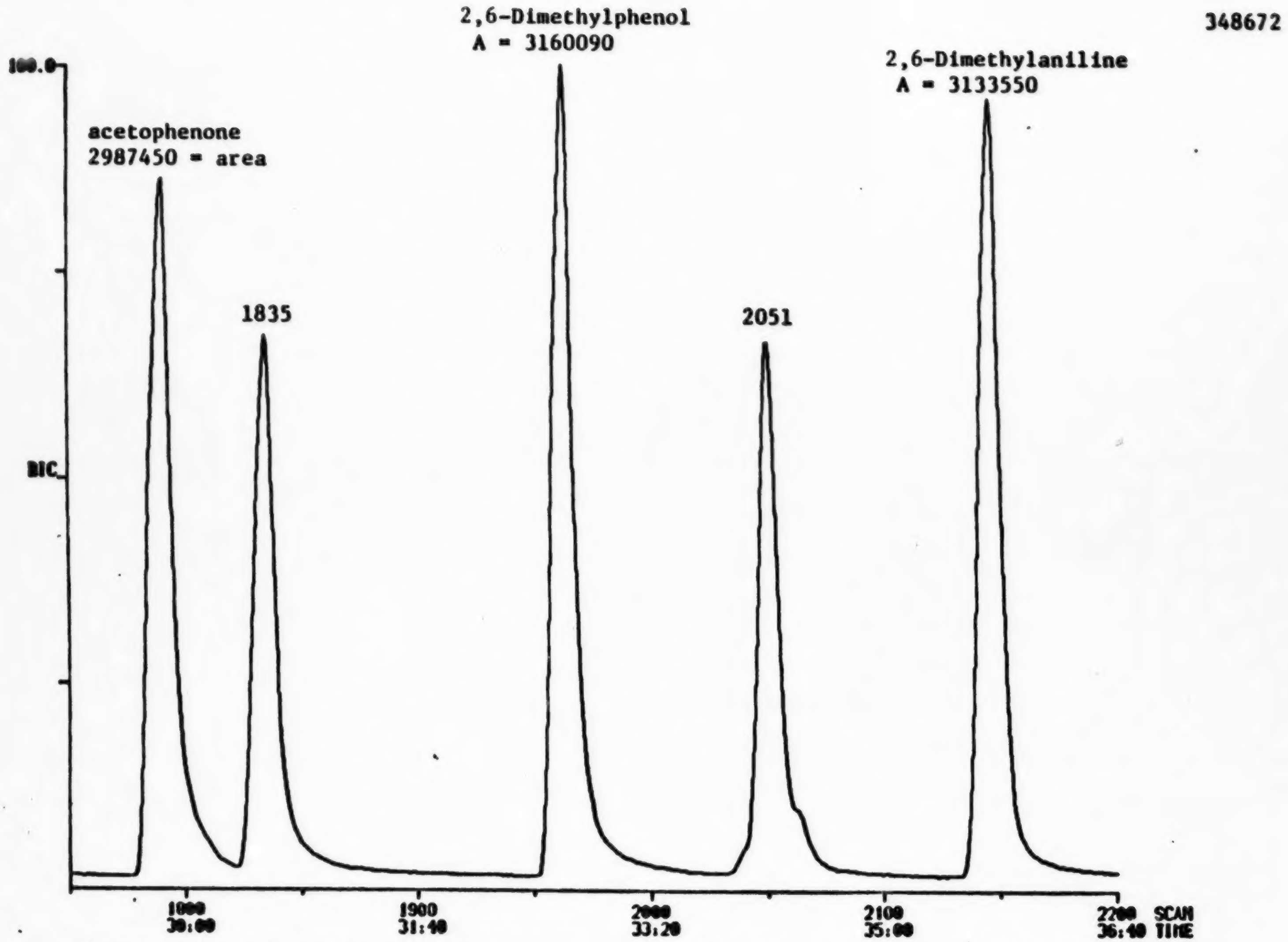


Figure 13.2. Reconstructed total ion current profile for acetophenone, 2,6-dimethylaniline and 2,6-dimethylphenol used for measuring acidity/basicity.

where

D = the distance between two peaks, measured between perpendiculars dropped from the peak apices

W_1, W_2 = widths at 1/2 height

Typically two hydrocarbons are used for this measurement (e.g., eicosane and heneicosane in Table 13.1). The specific ions of the two components in the SPS to be used for measurement of the SN and the acceptable criteria are given with each analytical protocol (e.g., see Table 13.6). An example of this measurement is depicted in Figure 13.3.

13.4.1.5 Resolution--

An example of calculating peak resolution or percent valley is shown in Figure 13.4. Minimal criteria and specific compounds used for this measurement are given in each analytical protocol. Percent valley is defined as:

$$\% \text{ valley} = \frac{\text{valley}}{\text{peak height}}$$

where

valley = height of valley relative to height of first peak

peak height = height of the first peak of the doublet.

13.4.1.6 Inertness--

Inertness of the chromatographic system's interface is determined from the methyl stearate peak. Methyl stearate ion ratios ($\underline{m}/\underline{z}$ 74 to 298; see Table 13.6) as well as the acidity/basicity ratios (Section 13.4.1.3) are used to assess system inertness.

13.4.1.7 Changes in Column Polarity and/or Stationary Phase Film--

Retention time shift (absolute or relative) of components in the SPS (for example, eicosane and heneicosane in Table 13.1) is used to determine deterioration of chromatographic performance as a capillary column ages from usage. Exact guidelines for acceptability are GC/MS system-dependent and must be established by the analyst for his own system. This change may reflect an alteration in polarity or stationary film thickness. The performance criteria are given in each analytical protocol.

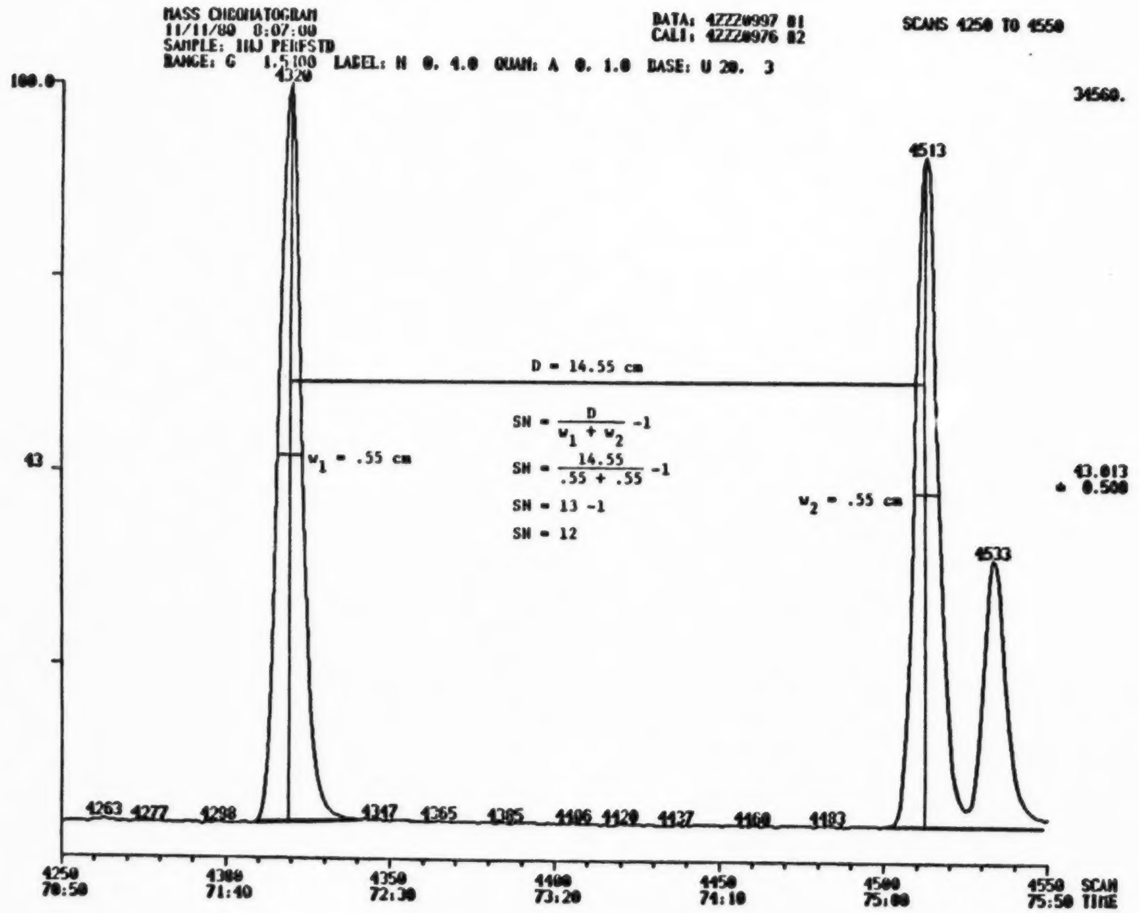


Figure 13.3. Extracted ion current profile of m/z 43 of eicosane and heneicosane used for measuring SN.

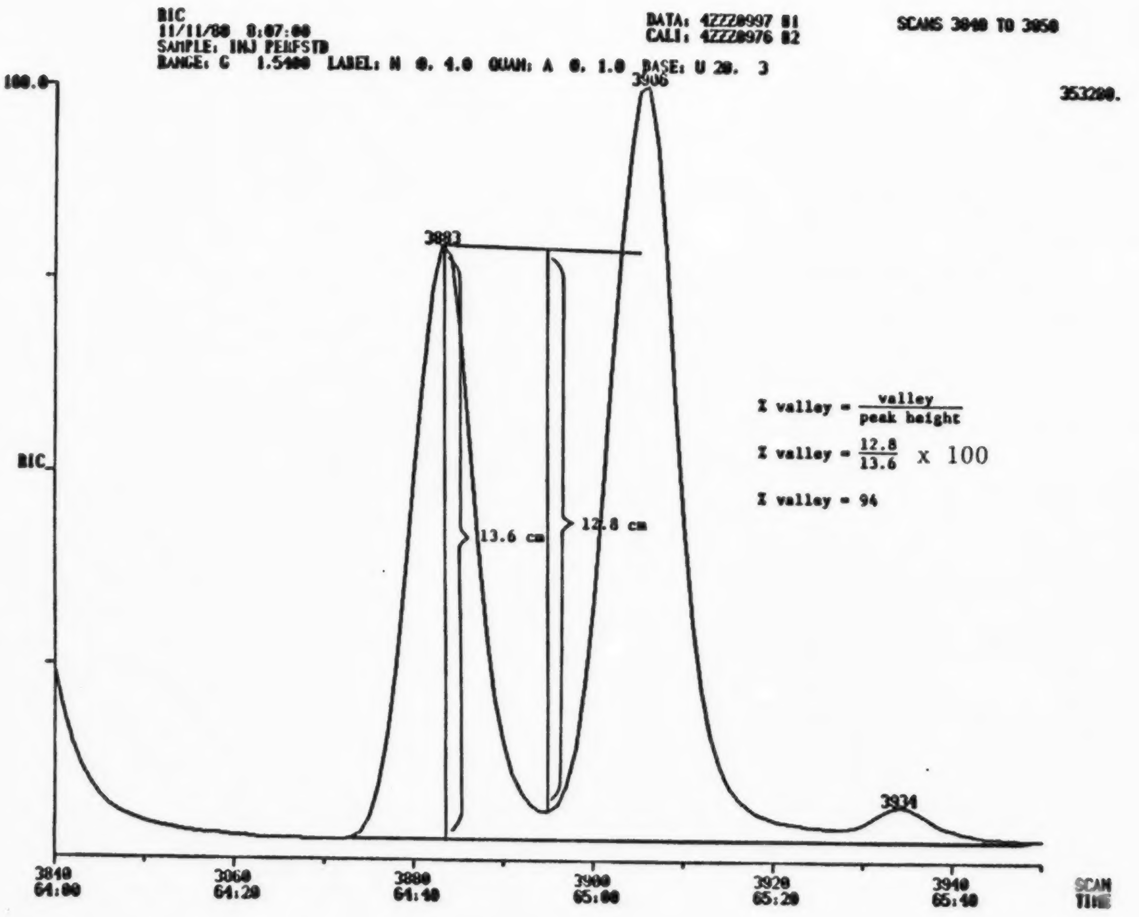


Figure 13.4. **Reconstructed total ion current profile of octadecene and octadecane used for measuring resolution** (percent valley).

13.4.1.8 GC/MS/COMP System Sensitivity and Dynamic Range--

Two components in each SPS are designated at levels which provide, upon analysis, information concerning sensitivity of the GC/MS/COMP system and potential overloading of the capillary column (dynamic range of analysis). The compounds selected, for example, methyl stearate and methyl decanoate (Table 13.1), are intended to be representative of the types of functionalities present in the extract/fraction to be analyzed. Example charts are provided in Table 13.6 for limit of detection (system sensitivity) of methyl stearate and capillary capacity (% PAF) for methyl decanoate. Specific criteria are given with each analytical protocol.

13.4.1.9 Presentation of System Performance Information--

Table 13.6 is a hypothetical example of the formatted information acquired from the SPS designated in the "extractable acid" (ESSA) analytical protocol. The information acquired is displayed for historical reference over a period of 14 days. The analyst can not only assess prior to analysis whether performance specifications have been met for that day's operation, but can also determine trends in performance to anticipate potential problems in the near future. Blank forms such as used for Table 13.6, but tailored for each analytical protocol, are provided with each protocol; the analyst can duplicate them for his own use.

Adherence to acquiring and assessing the system performance information is an essential facet of the quality control procedures in the MAS.

13.4.1.10 Failure to Meet Chromatographic Criteria--

A new capillary column should be well within the specifications provided for each analytical protocol's SPS. When one or more of the criteria can no longer be met, the capillary column should no longer be used. Rejuvenation may be attempted by removing 2 coils from each end of the capillary. If the coils are removed from a glass (not fused silica) column, the ends must be straightened, and the straightened ends should be recoated with a 0.1% Carbowax 20M/methylene chloride solution (Section 13.3.2).

13.4.2 Sample Analysis

After establishing that the MS tuning and chromatographic criteria have been met, analysis of sample extracts may begin. The analysis conditions for each sample extract are prescribed in each analytical protocol and are identical to those for the SPS. If more than one day's GC/MS running time is required for a set of sample extracts from an analytical protocol, the system performance solution must again be analyzed as the first analysis of a new day.

When sample extracts from a different analytical protocol are to be analyzed, then the operating conditions prescribed for that protocol are to be set up and the system performance solution for that protocol analyzed first.

A set of sample extracts is defined as extracts derived from field samples, surrogate samples, blanks, controls, standards used for retention time and RMR determination, etc., that are all associated with a single analytical protocol. The recommended analysis order is laboratory and field blanks and controls, surrogates and collected field samples, respectively.

13.4.2.1 Compound Identification--

At the Master Analytical Scheme user's discretion, the identities of the compounds in the sample extracts are established by computer search and/or manually. Table 13.7 gives suggested levels of confidence for the identifications, depending on the approach(es) employed.

13.4.2.2 Quantification--

13.4.2.2.1 General Description of Approach--The general approach to quantification employed by the Master Analytical Scheme is to determine the peak area ratios of the major ion(s) of the analyte relative to those of a deuterated internal standard and, using a known detector response ratio for the analyte and standard, known concentration of standard, and recovery factor for the analyte, calculate the amount of analyte present in the original water sample processed.

Once the analyst has identified the components, he may wish to use the relative molar response (RMR) factors supplied with the Master Analytical Scheme protocols (Appendix B) or determine his own response factors on the GC/MS/COMP system employed for sample extract analysis. The latter will be, of course, more accurate. In addition, the MAS user may frequently analyze for target compounds not in Appendix B, in which case he should

Table 13.7. SUGGESTED LEVELS OF CONFIDENCE FOR COMPOUND IDENTIFICATION

Level I	Computer Interpretation. The raw data generated from the analysis of samples are subjected to computerized deconvolution/library search. Compound identification made using this computerized approach has the lowest level of confidence. In general Level I is reserved for only those cases where verification of tentative compound identification is the primary intent of the qualitative analysis.
Level II	Manual Interpretation. The plotted mass spectra are manually interpreted by a skilled interpreter and compared to those spectra in a data compendium. In general a minimum of five to eight masses and intensities (±20%) should match between the unknown and library spectrum. This level does not utilize any further information such as retention time since, for many compounds, the authentic compound may not be available for establishing retention times.
Level III	Manual Interpretation Plus Retention Time/Boiling Point of Compound. In addition to the criteria attained under Level II, the retention time of the compound is compared to the retention time of a standard which has been derived from previous chromatographic analysis. Also the boiling point of the identified compound is compared to the boiling points of other compounds in the near vicinity of the one in question when a capillary coated with a non-polar phase has been used.
Level IV	Manual Interpretation Plus Retention Time and Matching Spectrum of Authentic Compounds. Under this level, the authentic compound has been chromatographed on the same capillary column using identical operating conditions and the mass spectrum and retention time of the authentic compound are compared to that of the unknown. Major peaks of the mass spectrum should correspond ±15% and retention times should correspond ±10%. The ultimate confirmation of identification consists of coinjection of authentic standard and observation of no change in mass spectrum or retention time.
Level IVa	Independent Confirmation Techniques. This Level utilizes other physical methods of analysis such as GC/Fourier transform IR, GC/high resolution mass spectrometry, or NMR analysis in the absence of authentic standard. This level constitutes the highest degree of confidence in the identification of organic compounds when no standard is available.

establish his own RMR data bank. To determine RMR factors, solutions containing precisely-known amounts of target compounds and standards must be analyzed in replicate. Compound concentrations and ion peak areas are obtained from the replicate analyses to allow calculation of RMRs.

The quantification process requires that the peak areas (or heights if areas are unavailable) of the ions listed for the analyte in the data bank (Appendix B) be determined. Generally two ions are listed. The second ion is provided in case the more intense ion is saturated, overlaps with an ion of the same mass from another eluent, or cannot produce an unambiguous area for some other reason. The peak areas for the internal standard ions listed are to be determined also. Retention times (as scan numbers) of the analytes and standard are to be recorded.

The quantification procedure is preferably to be performed by computer, and a computer program (MASQUANT) has been developed for this purpose. However, an understanding of the process used is essential and a detailed description is presented here. This section includes procedures for determination of response factors as well as for quantification of analytes.

13.4.2.2.2 Target Compound RMR Determination--If target compounds and internal standards have been analyzed as part of an SPS or for the purpose of determining response factors for a data base, the response factors on a molar basis (RMRs) are to be determined from equation 1.

$$RMR_{\text{target/standard}} \quad \frac{A_{\text{sought-for}} \cdot MW_{\text{sought-for}} \cdot g_{\text{standard}}}{A_{\text{standard}} \cdot MW_{\text{standard}} \cdot g_{\text{sought-for}}} \qquad \text{eqn. 1}$$

where

A = ion area (or height)

g = grams injected

MW = gram-molecular weight (if compounds are derivatized, use the molecular weight of the derivative).

Specific instructions for determining RMRs are included in each analytical protocol. Table 13.8 is a form that may be used for tabulating raw RMR data.

13.4.2.2.3 RMR Correction Factors--If the tune of the instrument when the samples are analyzed differs from the tune of the instrument when

Table 13.8. RMR RAW DATA - MAS

Date(s): ______________ **Run identification code:** ______________

MAS fraction: ______________________________

GC column and program: ______________________________

Notebook reference: ______________________

Compound	MW[a]	Amount (ng)[b]	Micro-moles	Ion	Area ( )[c]	Area ( )	Area ( )	Area ( )

[a] In case of derivative, use MW of derivative.

[b] ng of material in solution ready for GC-MS.

[c] Designate code number of each GC-MS run; if run on different dates, give dates.

the RMRs were determined, errors will be introduced into the quantification. With a change in tune, the historical RMRs are no longer correct when applied to the calculation of compound quantities. Depending on the exact nature and magnitude of the tuning variance, the effect on the RMRs can range from insignificant to order-of-magnitude. An empirically derived method of determining a correction factor which will reduce (not eliminate) these errors has been developed. It is suggested that this correction factor be applied if the relative abundances of the major ions of PFT or DFTPP observed during the analysis of the SPS differ by more than ±15% from those observed when the RMRs were determined. This figure is currently an arbitrary figure and may be raised or lowered at the discretion of the analyst. For this reason the relative abundances for the major ions of PFT and DFTPP observed when the MASQUANT data base historical RMRs were determined are given in Appendix B.

RMR data obtained from the analysis of internal and external standards in the SPS solution may be used to correct for tuning differences, and, therefore, RMRs, between the current and historical data file (Appendix B). The current RMRs are calculated from selected ion peak areas of the internal and external standards using equation 1, and tabulated on forms provided in each analytical protocol (see Table 13.9, for example). The RMRs determined under the different tuning conditions can then be correlated using a linear regression analysis. To determine the slope and intercept for the linear regression, the series of current RMRs for each standard ion of the internal and external standards (Table 13.9, for example), are compared to the RMRs in the Appendix. (The Appendix contains tables of the matrix

Table 13.9. GC-MS SYSTEM PERFORMANCE TEST: RMR CHECK FOR SEMIVOLATILE STRONG ACIDS (ESSA)

Date: ____________________ Run ID Code: ____________________

DATA

Standard	MW	Amount (ng)	μM	Ion (m/z)	Area (Run 1)	Area (Run 2)
4-Fluoro-2-Iodo-toluene (FIT)	236			109	49,903	49,351
				236	63,372	64,051
d_{13}-heptanoic acid	143			77	46,584	56,538
				91	13,314	15,465
d_5-benzoic acid	127			82	28,756	31,509
				110	57,351	61,935

MATRIX OF STANDARD ION RMRs

Standard	Ion	109	236	77	91	82	110
FIT	109	-	0.78	2.77	9.56	3.51	1.77
	236	1.28	-	3.55	12.25	4.50	2.27
d_{13}-heptanoic acid	77	0.36	0.28	-	3.47	1.26	0.64
	91	0.10	0.08	0.29	-	0.37	0.19
d_5-benzoic acid	82	0.28	0.22	0.79	2.72	-	0.51
	110	0.56	0.44	1.56	5.39	1.98	-

$$RMR_{x/y} = \frac{A_x \cdot MW_x \cdot ng_y}{A_y \cdot MW_y \cdot ng_x}$$

A = ion area or height
ng = ng injected
x = analyte
y = standard

COMMENTS:

of historical standard ion RMRs.) A linear regression analysis of the current responses as y versus the corresponding historical responses (Appendix) as x is performed. The slope (m) and intercept (b) are used to modify the historical (data bank) RMRs for current use by the equation:

$$y_{(\text{RMR today})} = mx_{(\text{data bank RMR})} + b$$

An example for one standard ion of the sample follows:

The Appendix gives the matrix of standard ions for the ESSA fraction when the historical RMRs were determined (Finnegan 4021). Table 13.9 gives the RMRs of the ions of the internal and external standards relative to one another observed in the SPS for ESSA run on a typical day. The linear regression analysis of the SPS RMR values of the internal standards relative to m/z 236 of FIT as $\underline{y}$ versus the corresponding historical RMR values from the Appendix as $\underline{x}$ gives a correlation coefficient of 0.995 with a slope of 1.31 and an intercept of -0.13. Thus, an RMR value of 0.81 from the data bank (Appendix) would be corrected as follows:

$y = mx + b$

$x = 0.81$

$m = 1.31$

$b = -0.13$

$y = 1.31\ (0.81) + -.13$

$y = 0.93$ (corrected RMR)

Several caveats should be mentioned concerning the use of correction factors: 1) as the correlation coefficient decreases from 0.9999...9 the accuracy decreases; 2) the fewer the x,y pairs the less accurate the correction factor (a minimum of 5 pairs is recommended); 3) this correction factor is meant to help reduce the error in RMRs and is only an alternate technique to determining the RMRs interspersed with samples. The latter approach is more accurate but may not be practical with very complex samples and an extensive list of sought-for compounds. The MAS computer program MASQUANT includes a subroutine to perform the linear regression analysis and calculate the corrected RMR using x and y data provided by the analyst.

13.4.2.2.4 Quantification of Compounds Not In MASQUANT Data Base--In most sample extracts there will be detected and identified components that are not in the MASQUANT data base, or even in the user's own special data bank, and for which there are no RMR or recovery data. To obtain an estimate of concentration, which would be accurate to within a factor of ten in most cases, the RMR and recovery factors of a structurally similar compound in the data base may be used.

13.4.2.2.5 Determination of Analyte Concentration--A flow diagram depicting the steps in this procedure is given in Figure 13.5. The following steps are performed:

(1) The amount of internal standard (I.S.) added to the initial volume of water sample multiplied by the fraction of the initial

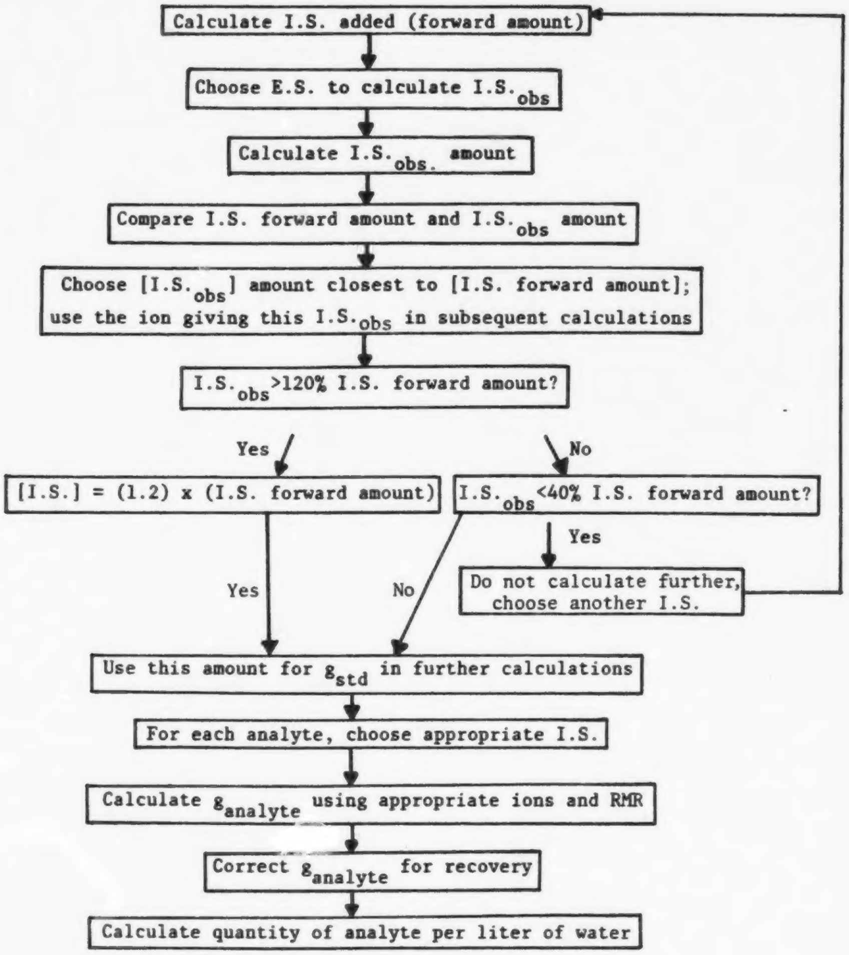

Calculate I.S. added (forward amount)
Choose E.S. to calculate I.S.$_{obs}$
Calculate I.S.$_{obs.}$ amount
Compare I.S. forward amount and I.S.$_{obs}$ amount
Choose [I.S.$_{obs}$] amount closest to [I.S. forward amount]; use the ion giving this I.S.$_{obs}$ in subsequent calculations
I.S.$_{obs}$>120% I.S. forward amount?
Yes
No
[I.S.] = (1.2) x (I.S. forward amount)
I.S.$_{obs}$<40% I.S. forward amount?
Yes
Do not calculate further, choose another I.S.
Yes
No
Use this amount for g_{std} in further calculations
For each analyte, choose appropriate I.S.
Calculate $g_{analyte}$ using appropriate ions and RMR
Correct $g_{analyte}$ for recovery
Calculate quantity of analyte per liter of water

Figure 13.5. Schematic of steps in quantification procedure. (obs = observed; E.S. = external standard; and I.S. = internal standard)

water analyzed is corrected for historical recovery by multiplying by the recovery factor in the Appendix and is called the forward amount.

(2) If more than one external standard (E.S.) is used, the external standard eluting in closest proximity to each internal standard is determined from the ratio of the spectrum number of the internal standard to the spectrum number of the external standard.

(3) The amount of internal standard recovered, relative to the external standard, is calculated on the basis of equation 2 which is derived from equation 1. Standard ion RMRs are given in each analytical protocol and the Appendix.

$$g_{int.std.} = \frac{A_{int.std.} \cdot MW_{int.std.} \cdot g_{ext.std.}}{A_{ext.std.} \cdot MW_{ext.std.} \cdot RMR_{int.std./ext.std.}} \qquad \text{eqn. 2}$$

where

$g_{int.std.}$ = grams of internal standard recovered from the amount of water processed
$A_{int.std.}$ = area of the ion peak selected for the internal standard
$MW_{int.std.}$ = molecular weight of the internal standard (of the derivative if the compound is derivatized)
$g_{ext.std.}$ = grams of external standard added to the extract immediately before GC/MS analysis
$A_{ext.std.}$ = area of the ion peak selected for the external standard
$MW_{ext.std.}$ = molecular weight of the external standard
$RMR_{int.std./ext.std.}$ = RMR of the internal standard ion relative to the external standard ion

From two to four calculated amounts can be generated depending on the number of standard ions used in the external and internal standards. Only ions that clearly originate with the standard under consideration should be used for calculations; avoid interferences.

(4) The calculated amount closest to the forward amount is selected. This amount and the ion of the internal standard giving rise to this amount are used in the quantification of the analytes.
   (a) If the calculated amount exceeds the forward amount by 21% or more, 1.2 times the forward amount is used. (Internal standard recovery has been observed to be 80-120%. If the calculated amount is outside this range, this standard should not be used to calculate analyte quantities because it has not been recovered properly.)
   (b) If the calculated amount is less than 40% of the amount of internal standard added, this internal standard should not be used for quantification of the analytes because of poor recovery. Calculate recovery of another internal standard.

(5) For each analyte the internal standard (giving adequate recovery) eluting in closest proximity is determined from the ratio of the spectrum number of the internal standard to the spectrum number of the analyte.

(6) The analytes are quantified from equation 3, derived from equation 1.

$$g_{analyte} = \frac{A_{analyte} \cdot MW_{analyte} \cdot g_{int.std.}}{A_{int.std.} \cdot MW_{int.std.} \cdot RMR_{analyte/int.std.}} \qquad \text{eqn. 3}$$

where

$g_{analyte}$ = grams of analyte recovered from the amount of water processed
$A_{analyte}$ = area of the ion peak selected for the analyte
$MW_{analyte}$ = molecular weight of the analyte (of the derivative if the compound is derivatized)
$g_{int.std.}$ = grams of internal standard recovered from the amount of water processed
$A_{int.std.}$ = area of the ion peak selected for the internal standard
$MW_{int.std.}$ = molecular weight of the internal standard (of the derivative if the compound is derivatized)

$RMR_{analyte/int.std.}$ = RMR of the analyte ion relative to the internal standard ion.

The base peak of the mass spectrum of the analyte is the ion of choice to use in quantification. The ion of the internal standard as well as the $g_{int.std.}$ to use have been determined in steps 1-4. The RMR to use is given in the Appendix or determined.

(7) The calculated $g_{analyte}$ (which is the amount calculated for the volume of sample processed) is corrected for recovery by dividing by the recovery factor listed in the Appendix. (If the listed recovery factor is 0.4 or less, the compound should be analyzed by a more appropriate protocol (e.g., ESSA rather than WABN).

(8) The amount of analyte corrected for recovery is divided by the amount of water analyzed in liters to yield the amount per liter.

An example of the steps involved in a typical calculation for an analyte in the ESSA fraction is given in Table 13.10. The computer software program (MASQUANT), however, executes these steps as the input information is provided via a dialogue. Appendix C gives the operating instructions for use of the computer program. Forms are provided in each analytical protocol for logging the raw GC/MS data necessary for calculation of analyte concentration.

Table 13.10. STEPS INVOLVED IN A TYPICAL QUANTITATIVE CALCULATION

Step	Data
(1) Analyze sample (see analytical protocol for conditions). In this example, a 200 mL water sample was collected and processed through the protocol.	Results:

Standards	MW	ng	Spectrum No.	Ion	Area
4-Fluoro-2-iodo-toluene (external)	236	198	878	109	30,065
				236	17,990
d_5-Benzoic Acid, Methyl Ester (internal) (data bank recovery = 0.92)	141	250	734	82	19,354
				110	39,349

$RMR_{82/109} = 0.28$ $RMR_{82/236} = 0.22$

$RMR_{110/109} = 0.56$ $RMR110/236 = 0.44$

Analyte	MW	Spectrum No.	Ions	Area
p-Toluic Acid, Methyl Ester (data bank recovery = 0.91)	150	1044	119	77,374
			150	27,412

Step	Data
(2) Determine amount of internal standard from ion areas, external standard amount (198 ng) and RMRs, using eqn. 2.	d_5-benzoic acid, methyl ester has two ions and 4-fluoro-2-iodotoluene has two ions. Therefore, there are four answers:

$$g_{I.S.} = \frac{A_{I.S.} \cdot MW_{I.S.} \cdot g_{E.S.}}{A_{E.S.} \cdot MW_{E.S.} \cdot RMR_{I.S./E.S.}}$$

m/z 82 and m/z 109 = 131 ng
m/z 110 and m/z 109 = 172 ng
m/z 82 and m/z 236 = 222 ng
m/z 110 and m/z 236 = 287 ng

(continued)